

# TCR usage in naive and committed alloreactive cells: implications for the understanding of TCR biases in transplantation

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The direct pathway of allorecognition is involved in acute allograft rejection and is characterised by TCR-mediated recognition of the MHC framework; this is thought to occur in a peptide-dependent but not peptide-specific manner. In contrast, the indirect pathway is restricted to the recipient's own MHC molecules and prevails in chronic rejection. In this pathway, the peptide has a major influence on the TCR recognition and selects alloreactive T cells with altered TCR V $\beta$  usage. However, qualitative analysis of V $\beta$  usage alone might limit our understanding of alloreactivity. The advantages of a combined quantitative assessment of V $\beta$  mRNA usage are discussed.

## Addresses

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## Abbreviation

APC antigen-presenting cell

## Introduction

The TCR is the antigen-receptor on mature T cells; it is a heterodimeric structure of  $\alpha$ - $\beta$  chains that interacts with peptides presented by MHC class I or class II molecules, depending on the route by which antigenic proteins are processed. The repertoire of mature T cells results from a complex process of  $\alpha/\beta$  TCR gene rearrangements and  $\alpha$ - $\beta$  chain associations that can theoretically generate a huge diversity of TCR specificities ( $10^{15}$  in the human). This diversity is ultimately restricted to a much lower figure in the blood of an adult individual (approximately  $25 \times 10^6$ ), as deduced from CDR3 sequences observed in analyses of several given, rearranged V $\beta$  families (with various CDR3 lengths) that are associated with given V $\alpha$  families [1•]. However, the understanding of the actual TCR repertoire diversity must also take into account the degeneracy (i.e. flexibility) of the recognition specificity — the same heterodimer is able to interact with many different peptides presented by the same MHC molecule (see [2] for a review). On the other hand, discrete changes in the sequence/polarity of the peptide recognised can cause different signaling in the T cell. Therefore, this multilevel complexity makes it somewhat difficult to calculate the precise meaning of repertoire size at the actual recognition level.

In addition, there is compelling evidence that only T cells recognising a sufficiently high number of MHC-peptide

complexes on activated antigen-presenting cells (APCs) would be allowed to undergo proliferation and differentiation. These are the major concepts to keep in mind when interpreting the data derived from the study of the T cell repertoire in normal and pathological conditions and, in particular for this review, following transplantation.

Uniquely, transplantation immunity involves 'unphysiological' TCR-MHC interactions superimposed on the normal mechanisms of immunity. As well as the effects of host MHC polymorphism, transplant immunologists studying the TCR repertoire in allorecognition must integrate the capacity of the TCRs of a high proportion of naive T cells (about 1%–7% [3–5]) to engage foreign allogenic-MHC-peptide complexes. This interaction (referred to as the direct allorecognition pathway), which 'contradicts' to some extent the MHC restriction of T cell recognition, probably stemmed from the governing principle of MHC-driven T cell positive-selection by thymic epithelial cells in the absence of further negative selection for allo-MHC-peptide complexes; thus allo-MHC recognition may result from cross-reaction with self-MHC expressed in the thymus.

In this short review, we focus only on new findings in this field. Besides what we have learnt from repertoire alterations in transplantation immunity during this past decade, recent work has shown that, in contrast to membrane expression of TCR chains, TCR mRNA is specifically upregulated by antigen ligands [6••]. Therefore, quantitative analysis of TCR mRNA accumulation represents a new approach for the study of T cell function.

## The molecular nature of direct allorecognition

In transplantation, reactive T cells can be activated by the recognition either of peptides derived from allogenic molecules and presented on self-MHC (i.e. the indirect pathway) [7] or of donor MHC-peptide complexes on allogenic APCs (i.e. the direct pathway) [8]. There are several lines of evidence that suggest prominent interactions of the TCR with the allogenic-MHC framework-determinants in the direct pathway [9]. Recently, Sebille *et al.* [10] reinforced this idea by observing that the direct presentation pathway was not associated with skewing of the CDR3 length distribution. This is in agreement with crystallographic studies, which show that — in contrast with self-restricted recognition — in direct allorecognition, most of the binding energy is dependent on TCR interactions with MHC helices, whereas contacts with peptide side-chains determine the specificity of the response [11]. In addition, it has been demonstrated that the peptide-specificity of a T cell response consistently decreased when donor and stimulator MHC-haplotype sequences

diverged. Indeed, a single variation in an MHC determinant directly contacting the TCR is sufficient to generate the majority of peptide-independent alloreactive clones [12\*\*]. Therefore, allogeneic interactions might be less sensitive to peptide variations than self-restricted ones, illustrating the degeneracy of direct allorecognition [13].

One of the most suitable models for the study of interactions with non-self MHC molecules has been the TCR 2C clone, derived from alloimmunisation of H-2<sup>b</sup> mice with H-2<sup>d</sup> cells. Interestingly, this clone displays recognition of both allogeneic (H-2L<sup>d</sup>)-restricted and self (H-2K<sup>b</sup>)-restricted ligands, allowing the comparison of these two types of interaction involving the same TCR. Whereas the two TCR–ligand complexes appear to have similar three-dimensional features, the relative influence of TCR residues on binding energy to allo- or self-ligand is different [14\*\*].

However, the influence of the peptide in the direct pathway of allorecognition remains questionable. Indeed, as demonstrated by Kovalik *et al.* [15], among T cell clones derived from mice sensitised by allogeneic spleen cells (H-2<sup>b</sup>), only 9% recognised APCs from allogeneic H2-DM<sup>-/-</sup> mice (H-2<sup>b</sup>), which present only the non-immunogenic CLIP peptide, and are thus peptide-independent. Moreover, alloreactive clones generated in this study exhibited a 10-times lower activation threshold than self-restricted clones, suggesting that high affinity could be a general feature of alloreactive clones. In addition, the fact that cardiac allografts from H2-DM<sup>-/-</sup> mice are not rejected earlier than those from MHC-class-II<sup>-/-</sup> mice reinforces the idea that the presence of donor MHC molecules is not sufficient to elicit a strong direct alloresponse and that the presence of antigenic peptides is required to trigger allograft rejection even if it is mediated by the direct presentation pathway [16\*].

### The contribution of direct and indirect pathways of allorecognition

The percentage of naive T cells responsive to alloantigen *in vitro* and *in vivo* is much higher than the percentage of T cells that respond to a nominal antigen. However, assessment of the alloreactive T cell precursor pool-size depends on the model used for its measurement [5]. Nevertheless, it has been shown, *in vitro*, that a high proportion of T cells are involved in the direct recognition event and that T cells activated by this pathway span a large part of the V $\beta$  transcriptome [10]. In a model of skin allografting in mice, direct-pathway T cells have been estimated to represent >90% of the T cell repertoire involved in the process of acute rejection, whereas in the indirect pathway alloreactive T cells would represent only 1%–10% of the cells involved [17]. Recently, Pietra *et al.* [18\*\*] showed unambiguously that CD4<sup>+</sup> alloreactive T cells are both necessary and sufficient for mediating acute cardiac allograft rejection through the direct pathway. Transfer of naive CD4<sup>+</sup> T cells also triggered rapid acute cellular rejection of accommodated xenografts [19].

In contrast, the indirect pathway promotes the development of chronic rejection [20]. Distinct clones, utilising a variety of self-MHC-restricted TCR V $\beta$  chains, can be involved against a single indirect allo-epitope mediating transplant rejection [21]. The specificity of this T cell response to donor antigens can be modified during the progression of rejection owing to intramolecular epitope spreading [17,20,22]. This causes continuous activation of naive T cells by new epitopes, and the resulting graft lesions may not require a high frequency of each clone in the successive waves of infiltrating cells [20]. T cell responses directed to minor histocompatibility antigens, such as tissue-specific antigens, can also be involved in allotransplant rejection [23,24]. Moreover, autoreactive responses might perpetuate and amplify the immune destruction of the transplanted organs [25\*,26,27].

However, whether the indirect allorecognition can also be sufficient to promote acute rejection remains controversial [20,28]. One of these recent studies [28], showing that incompatibility of a minor antigen such as H-Y — which is strictly recognised through indirect recognition — triggers an acute skin rejection, remains difficult to interpret and to reconcile with ‘physiological’ conditions that actually prevail *in vivo* in nontransgenic animals. Indeed, the use of transgenic TCRs provides a highly artificial frequency of indirect-pathway-dependent clones which, in fact, mimics the situation that only the direct pathway can match.

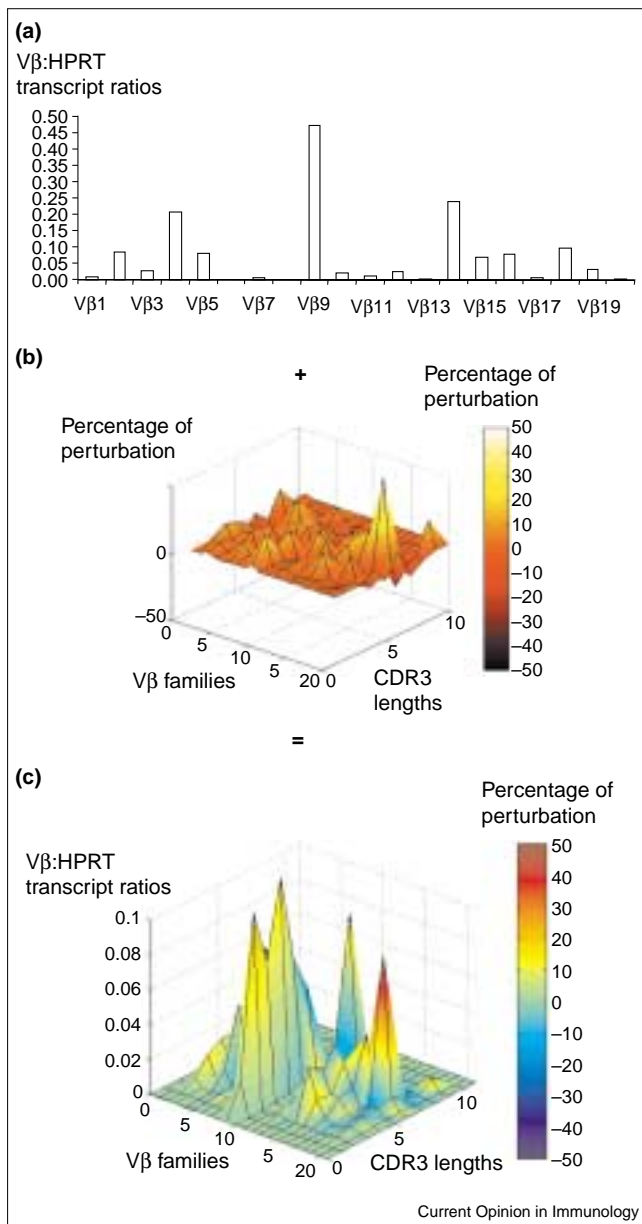
### V $\beta$ usage in allorecognition

Although only the cloning and sequencing of all altered CDR3s would confirm a clonal response [29], even a particular TCR sequence can to some extent recognise different peptides [2]. Analysis of the TCR repertoire has been essentially based on the study of the CDR3 length distribution [30,31] in peripheral and graft-infiltrating T cell populations — only stereotyped V $\beta$ J $\beta$  rearrangements are usually cloned and sequenced. Using this read-out, allospecific T cell populations infiltrating allografts have been shown to display a restricted repertoire (for a review, see [32]). For instance, Slachta *et al.* [33] found sequences of identical  $\beta$ -chain TCR transcripts clonally expanded in different histological sites and at different times, when cardiac allografts were harvested from patients with chronic rejection.

Likewise, the TCR V $\beta$  repertoire in humans receiving a pre-heart-transplant transfusion was reduced in both the CD4<sup>+</sup> and CD8<sup>+</sup> T cell compartments for between <1 month and 18 months, with more pronounced changes within the CD8<sup>+</sup> T cell subset [34].

Only few oligoclonal expansions (involving the V $\beta$ 2 and V $\beta$ 13 families) were recurrently found in a model of acute cardiac allograft rejection in MHC-mismatched rats [35]. In the same combination, alloreactive CD8<sup>+</sup> T cells bearing a public rearrangement (V $\beta$ 18D $\beta$ 1J $\beta$ 2.7) were found in the phase of donor-specific tolerance induction and probably contributed

Figure 1



Ways of studying TCR Vβ usage. This can be assessed by quantitative (a), qualitative (b) or combined qualitative/quantitative (c) methods. HPRT (hypoxanthine phosphoribosyl transferase) is a 'housekeeping' gene. Percentages of perturbation are obtained by comparing the distribution profiles of CDR3 lengths in controls (which are Gaussian) and in the samples studied. Whereas qualitative analyses have been widely used through Immunoscope® and Reperturb® (b), several quantitative methods have recently emerged. (c) Gives an example of the approach combining qualitative (color code) and quantitative (Z axis) analysis of Vβ chain usage at the CDR3 length distribution level. These images are obtained from an arbitrary example. For Vβ transcriptome landscapes during direct and indirect pathways of allorecognition, see [10].

to tolerance [29]. Indeed, an anti-TCRVβ18Dβ1Jβ2.7 DNA vaccination abolished tolerance induction [36], whereas anti-TCRVβ13 DNA vaccination led to a significant prolongation of allograft survival [37].

TCR alteration can be observed in several 'active' immune processes, including tolerance. Recently, in kidney allograft models in swine, Baron *et al.* [38] observed a clonal dominance of T cells bearing the Vβ100+ family, 30 days following the grafting. Likewise, in humans, αβ T cells infiltrating chronically rejected kidney allografts exhibit an altered TCR Vβ usage when it is associated with acute lesions [39]. In contrast, in the same study, transplants presenting as 'chronic' rejection with no evidence of acute superimposed lesions using histological Banff grading (i.e. without tubulitis or acute vasculitis) displayed resting-type, Gaussian TCR profiles [39]. The fact that organs with a histology typical of 'chronic rejection' do not exhibit an altered TCR pattern suggests that they are not driven by chronic immunological insult.

On the whole, these recent examples showed altered repertoires in chronic rejection, probably reflecting recognition of peptides in a self-restricted (indirect) pathway. This suggests that it might be possible to modulate the alloresponse using peptides with antagonistic properties [13,40,41]. TCR-based immunotherapies could also be applied as long as particular Vβ families or CDR3 regions are involved [41,42]. However, due to the variety of MHC-restriction determinants that shape TCR usage among individuals differently, public TCR alterations are rare events [30]. In addition, some oligoclonal alterations can be observed in an apparently 'resting' T cell population, which should only present a polyclonal (i.e. Gaussian) CDR3 length distribution of Vβ families [30] and which includes naive T cells as well as a composite population of memory and regulatory T cells. This reinforces the need for cautious interpretation of alterations in an individual [30]. As an example, 58% of mice over the age of two years, and almost all humans over the age of 40, present CD8+ T cell clones that could be large enough to distort the otherwise quite predictable αβ TCR repertoire of the CD8+ T cells [43,44,45]. These clones can correspond to memory T cells or can be identified as virus-specific CD8+ T cells [46]. Heat-shock protein (hsp)-reactive lymphocytes are also involved in allograft rejection [47–49] and can contribute to the emergence of clonal expansions in the Vβ repertoire of T cells. Moreover, the repertoire of T cells in normal human skin is apparently subjected to environment-specific selection throughout life [50]. Absence of Gaussian CDR3 distribution patterns of length has been also found in the gut [30]. Interestingly, γδ T cells of the skin and the intestine also express an oligoclonal repertoire [51]. In addition, Attuili *et al.* [52] showed that each individual mouse expresses a preimmune repertoire, with distinct antigen specificities and with corresponding expanded clones. Likewise, subtle biases in the naive TCR repertoire can significantly impact the composition, specificity and strength of the T cell response to a given peptide [53–56].

Finally, prominent interactions of the TCR with the MHC helices seem to affect the direct pathway of allorecognition

more than peptide residues (see above), as suggested from direct mixed-lymphocyte reactions [10] and unmodified acute rejection (M Guillet *et al.*, unpublished data). Indeed, in this case, qualitative repertoire analyses would not be efficient enough to detect T cells activated through the direct recognition pathway, which accordingly would occur in the absence of skewing in the CDR3 length distribution, since allo-MHC recognition probably depends on recognition of framework determinants rather than the peptide itself [10]. Thus, our understanding of the biological relevance of TCR repertoire alterations would be enhanced by knowledge of the amounts of mRNA involved but this has been rarely assessed and, when it has it was for specific families only [35].

### Quantitative assessment of TCR V $\beta$ usage

Recently, several groups reported quantitative analyses of V $\beta$  usage. Using an RT-PCR–ELISA technique, Lennon *et al.* [6\*\*] showed that TCR engagement selectively increases V $\beta$  gene transcription. Yoshida *et al.* [57], using an adaptor-ligation PCR-based hybridisation assay, observed no correlation between MHC haplotypes and V $\beta$  and V $\alpha$  mRNA accumulation in splenocytes. Other groups recently proposed quantitative approaches to assess V $\beta$  transcript usage [58–63]. New techniques devoted to the study of TCR expression on the cell surface, based on the utilisation of anti-V $\beta$  monoclonal antibodies [64,65], emerged but they cannot be compared with the transcriptional assessment because of downmodulation of the TCR [66,67]. Indeed, when T cells are stimulated with antigen, engaged TCRs are internalised and degraded, resulting in greatly reduced surface TCR levels for up to several days post-stimulation [67,68], with comodulation of nonengaged TCRs [68].

Interestingly, Mannon *et al.* [69,70] observed *in vivo* that a substantial proportion of graft-infiltrating CD8<sup>+</sup> cells do not express detectable levels of TCRs on their cell surfaces. This CD8<sup>+</sup>TCR<sup>-</sup> population consists of CD8<sup>+</sup> T cells that have downregulated their cell surface TCR proteins. Studies of TCR internalisation within the peripheral compartment in V $\beta$ 5 TCR transgenic mice have led to the suggestion that the decrease of the signalling following TCR internalisation would result in the upregulation of the TCR gene recombination machinery, which could allow ‘receptor revision’ — a process by which T cells would attempt to express new TCR affinities/specificities [71\*\*]. Although TCR revision has not been yet established in non-transgenic organisms, this would cause further complexity in the interpretation of kinetic changes in TCR expression [71\*\*].

Due to all these limitations, we developed a new approach based on a combination of qualitative analysis of V $\beta$ -chain gene segment usage at the CDR3-length level and quantitative PCR assessment of all CDR3-length-restricted mRNAs in each V $\beta$  family. This global T cell mobilization can be represented as global ‘landscapes’ for each informative time-point of a T cell immune response to give a direct visualization of the level of mRNA concerned with any CDR3

biases of the V $\beta$  transcriptome (see Figure 1). This new approach, referred to as TcLand, allows us to study not only clonal TCR alterations, but also situations where no biases in CDR3 length distribution (or clonal responses) are detectable, such as when T cells have been stimulated with the superantigen TSST-1 or after Con-A stimulation [10]. Furthermore, using this approach, we showed that naive T cells vigorously accumulate V $\beta$  mRNA without alteration of CDR3 length when cocultured with allogeneic APCs [10]. Similar profiles are also observed in graft-infiltrating cells during acute rejection of MHC-incompatible heart allografts in the same combination (M Guillet *et al.*, unpublished data).

### Conclusions

TCR alterations during allograft rejection have been mostly studied in the circumstances of indirect pathway activation. However, distinguishing ‘resting’ from exhaustively activated populations and the possibility of multiple alterations within the ‘resting’ population make interpretation difficult. In addition, in the direct pathway, TCR recognition of alloligands involves more molecular interactions with the MHC framework determinants than with the peptide residues. These observations query the relevance of analyses restricted to qualitative TCR repertoire alterations. A combined quantitative and qualitative assessment of TCR mRNA accumulation — as in the TcLand approach — could improve the understanding of the immunological events taking place during rejection or tolerance of allografts.

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