Human T cell repertoire: what happens in thymus does not stay in thymus

Antonio La Cava

Commentary

The T cell receptor (TCR) repertoire is diverse, thus allowing recognition of a wide range of pathogens by T cells. In humans, the study of the formation of TCR repertoires is problematic because of the difficulty in performing investigations in vivo. In this issue of the *JCI*, Khosravi-Maharlooei and colleagues describe a new humanized mouse model that allows direct investigations on this topic. Using high-throughput and single-cell TCR–complementarity-determining region 3 β (TCR-CDR3β) sequencing, the authors were able to demonstrate that human thymic selection is a major driver of TCR sequence sharing, also implicating a preferential selection of shared cross-reactive CDR3βs during repertoire formation.

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Shaping the T cell repertoire

The generation of a broad variety of T cell receptors (TCRs) during T cell development in the thymus creates T cell repertoires capable of recognizing large numbers of antigens through multitudes of different TCRs. By recombination, random insertion, deletion, and substitution, the rather small set of genes that encode the TCR can create a great diversity, with the complementarity-determining region 3 (CDR3), which serves as the primary site of antigen contact, accounting for most of the variation within an individual's T cell repertoire. Moreover, this CDR3 diversity is primarily affected at the level of the β-chain (CDR3β) (1).

The host's T cell pool is shaped through a process by which TCR-expressing thymocytes undergo positive and negative selection of self-peptide–MHC complexes in the thymus, thereby generating naive T cells that are both self-tolerant and self-MHC restricted (2). However, a consequence of self-MHC restriction is that all peripheral T cells are essentially self-reactive, explaining why the peripheral T cell repertoire contains an elevated frequency of autoreactive T cells, even in healthy individuals (3). Incidentally, this aspect also creates grounds for the possible development of autoimmune responses that therefore need to be kept under constant control throughout life by multiple, often complementary, mechanisms (4).

Of note, although TCR gene rearrangement is believed to occur randomly, some clonotypes are more commonly produced than others (5), leading to unequal frequencies of naive T cell clonotypes and so-called "public" clonotypes that are shared among individuals (6). This difference in frequency distribution has been the subject of several investigations that have resulted in varied explanations but no consensus. As a result, although a dissection of the events that lead to the formation and diversification of the human TCR repertoire remains critical to understanding the mechanisms of T cell development and diversity, ethical constraints and lack of suitable models have complicated the study of the formation of human TCR repertoires in vivo.

New insights from a humanized mouse model

In this issue, Khosravi-Maharlooei and coworkers monitored TCR formation and outcomes in humanized mice that had been engrafted with human fetal thymus and human hematopoietic stem cells (HSCs) (7). This strategy allowed a recapitulation of critical mechanisms of in vivo formation of the human TCR repertoire under syngeneic and allogeneic conditions, mimicking the development of human thymocytes under conditions that are as close as possible to physiological settings. Through high-throughput and single-cell TCRβ-CDR3 sequencing analyses of human thymocytes in human thymi (and in the periphery) of humanized mice, the authors were able to accredit human thymic selection as a major driver of TCR sequence sharing and implicate a preferential selection of shared cross-reactive CDR3βs during repertoire formation. As such, this study by Khosravi-Maharlooei and colleagues delineates the impact of human thymic selection on shared TCR sequences and their presence among mature T cells.

Khosravi-Maharlooei and coworkers analyzed the formation of the human TCR repertoire under different conditions and compared data from three different groups of humanized mice. The first group of animals received the same fetal liver HSCs and autologous fetal thymus, resulting in the same genetic background and TCR selection in the same thymus. The second group of mice also had the same genetic background and same fetal liver HSCs, together with either an autologous fetal thymus or an allogeneic fetal thymus. This group of mice allowed evaluation of thymic selection in a different thymus, with the T cells in the grafted thymus derived from HSCs given intravenously. Finally, the third group of mice consisted of thymectomized animals transplanted with the same fetal liver HSCs and autologous fetal thymus. Cell sequencing was done for both

thymocytes and peripheral CD4+ and CD8+ T cells, and combined approaches ensured the elimination of possibly confounding preexisting thymocytes and their progeny from transplanted thymic tissue (8).

With this model, comparisons between replicate mice allowed Khosravi-Maharlooei et al. to discern whether exposure to the same antigens for positive and negative selection in the thymus from the same human donor leads to the formation of similar TCR repertoires in different individuals. The model also allowed examination of the effects of positive and negative selection on the human TCR repertoire and the analysis of V-J pairings, as discussed below.

The first notable finding was that replicate humanized mice generated divergent repertoires. Low clonality among different human thymi underscored the selection of highly diverse repertoires, and the divergence of TCRβ repertoires in thymocytes generated in identical thymi from the same HSCs reflected the stochastic nature of TCR repertoire formation. These analyses also showed that thymic selection narrowed the T cell repertoire and increased CDR3β sharing, with augmented clonality and a progressive decrease in diversity in conjunction with T cell maturation. Indeed, despite the stochastic nature of the process, thymic selection favored the selection of shared sequences between individuals (with a lower divergence at the amino acid level than at the nucleotide level). This result indicated that while stochastic formation of the repertoire results in differences between replicates, there was nonetheless a preferential selection of public sequences by disparate HLA alleles, which is in line with studies that have shown a similar overlap between TCR repertoires of monozygotic twins and TCR repertoires of unrelated individuals (9). Further analyses of the frequency of the shared CDR3βs at the double-positive thymocyte stage compared with single-positive and peripheral CD4+ and CD8+ T cells indicated that both positive thymocyte selection and negative thymocyte selection were responsible for the increased CDR3β overlap between individual human T cell repertoires.

Structurally, the shared CDR3βs were shorter in length because of fewer numbers of inserted nucleotides at the V-D

and D-J junctions. Moreover, CDR3β shortening continued further during the transition from thymic double-positive to single-positive to peripheral CD4+ and CD8+ T cells, consistent with past studies (10, 11). As CDR3β shortening within the most abundant, but not the less abundant, sequences accompanied the progression of thymic selection (CD69+ double-positive to single-positive transition), Khosravi-Maharlooei et al. suggested that shorter, more common CDR3βs could avoid negative selection and facilitate positive selection due to a lower affinity for cross-reactivity with diverse MHC peptides. Notably, shared CDR3βs (in single-positive CD4+ T cells) had different V gene, but not J gene, usage, with approximately 20% to 25% of shared CDR3βs using the same V-J pair. The same TCRβ chain almost always paired with different α chains, and thymic selection did not appear to influence the overall V and J gene usage.

Shared CDR3βs also displayed an increased likelihood of cross-reactivity as compared with unshared CDR3βs in mixed lymphocyte reactions (HLA-mismatched); therefore, Khosravi-Maharlooei et al. wondered whether those shared sequences might be enriched for autoreactivity. Taking advantage of data from the network for Pancreatic Organ donors with Diabetes (nPOD) program (12), the authors tested for autoreactivity by identifying shared sequences in type 1 diabetes–associated (T1D-associated) CDR3β and CDR3βs amino acid sequences reactive to islet self-antigens. The finding of higher T1D autoreactivity for the shared versus non-shared CDR3β sequences supports the possibility that autoreactive TCRs shared by different individuals are indeed preferentially selected. Also, the investigation of the amino acid usage at each position of the CDR3β suggests that selected shared sequences could have weaker interactions with self-peptides than unshared sequences. In particular, the increased hydrophobic amino acid usage at positions 6 and 7, which influence self-peptide–MHC complex reactivity during positive and negative selection (13), during human thymocyte positive selection was lost in the transition from single-positive thymocytes to peripheral T cells, i.e., after negative selection. Considering that positive selection favored shorter shared sequences with reduced hydropho-

bicity at positions 6 and 7 of the CDR3βs, the occurrence of weaker interactions with self-peptides (due to low affinity for self-peptide–MHC) for the shared versus unshared sequences could promote these sequences by facilitating their escape from negative selection (14).

Conclusions

In all, the study by Khosravi-Maharlooei and colleagues advances the current understanding of basic mechanisms of human TCR repertoire formation and T cell development. Human thymi formed varied TCR repertoires that diverged in the presence of the same genetic background. Thymic selection narrowed those TCR repertoires, promoting an overlap among human TCRβ sequences in which shared sequences had shorter CDR3β lengths and were cross-reactive and autoreactive. While previous work has suggested a role for recombination bias or convergent recombination in the generation of public sequences (15), the work of Khosravi-Maharlooei et al. delineates a role of human thymic selection in enriching public TCRβ sequences, in which distinctive characteristics of the CDR3βs could favor positive selection and escape negative selection.

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