THE MANY IMPORTANT FACETS OF T-CELL REPERTOIRE DIVERSITY

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In the thymus, a diverse and polymorphic T-cell repertoire is generated by random recombination of discrete T-cell receptor (TCR)-αβ gene segments. This repertoire is then shaped by intrathymic selection events to generate a peripheral T-cell pool of self-MHC restricted, non-autoaggressive T cells. It has long been postulated that some optimal level of TCR diversity allows efficient protection against pathogens. This article focuses on several recent advances that address the required diversity for the generation of an optimal immune response.

STRUCTURAL DIVERSITY
The availability of T cells that express a wide array of T-cell receptors (TCRs), specific for the same epitope, that differ from each other in the TCR segments used, primary and/or tertiary structure.

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Vigorous reactivity against pathogens a lack of overt reactivity to self are the defining features of a functional adaptive immune system. As postulated by the clonal selection theory[1], both are best achieved when lymphocytes express diverse, clonally distributed antigen-specific receptors. The immune system of vertebrates and, in particular, of mammals has evolved to potentially produce large numbers of diverse, clonally distributed antigen-specific receptors. Once the initial diversity is produced in primary lymphoid organs, further diversification occurs by somatic hypermutation of the B-cell receptor[2] and by functional diversification of effector T cells[3–6]. In the case of T cells, the protective immune response relies on the presence of a T-cell population that is poised to respond to peptides derived from pathogens, bound to self-MHC molecules. As the organism cannot predict the precise pathogen-derived antigens that will be encountered, the immune system relies on the generation and maintenance of a diverse T-cell receptor (TCR) repertoire. So, it follows that the size and diversity of the available naive T-cell repertoire are crucial in shaping the immune response to a given antigen.

TCR diversity is confined to the complementarity-determining regions (CDRs), the parts of the molecule that contact the peptide–MHC (pMHC) complex. The TCR–pMHC interaction has been reviewed at length[7,8] and is only briefly discussed here. The TCR-αβ — a heterodimer composed of α- and β- chains — uses its six flexible and hypervariable CDRs, three each from the α- and β- chains, to sense the composite surface of the uppermost parts of MHC α-helices and of the peptide that lies between them (FIG. 1). The markedly polymorphic CDR3-α and -β, which abound in junctional and non-germline variability, dominate in the recognition of peptide, although all CDRs can contact both peptide and MHC. Despite these general rules, many biological consequences of TCR–pMHC contact remain only partially resolved, including the structural nature of crossreactivity[9–11], the degree of TCR specificity/promiscuity and the nature of discrimination between closely related peptide ligands.

Defence against invading pathogens is the rationale of the immune system. It therefore stands to reason, that TCR diversification must have evolved to keep up with emerging pathogens, to cover most of the antigenic universe with corresponding receptors. Surprisingly, however, experimental evidence is still lacking on the qualitative and quantitative relationships between potential and actual lymphocyte diversity and the outcome of immune defence against pathogens; and on the extent to which variability in the naive repertoire affects the magnitude and complexity of antigen-specific immune responses.

This review addresses our knowledge of TCR-αβ lineage T-cell diversity in relation to immune defence and outlines some of the most important questions facing us. Two broad categories of T-cell diversity will be considered: first, STRUCTURAL DIVERSITY arising from different primary, secondary, tertiary and/or combinatorial

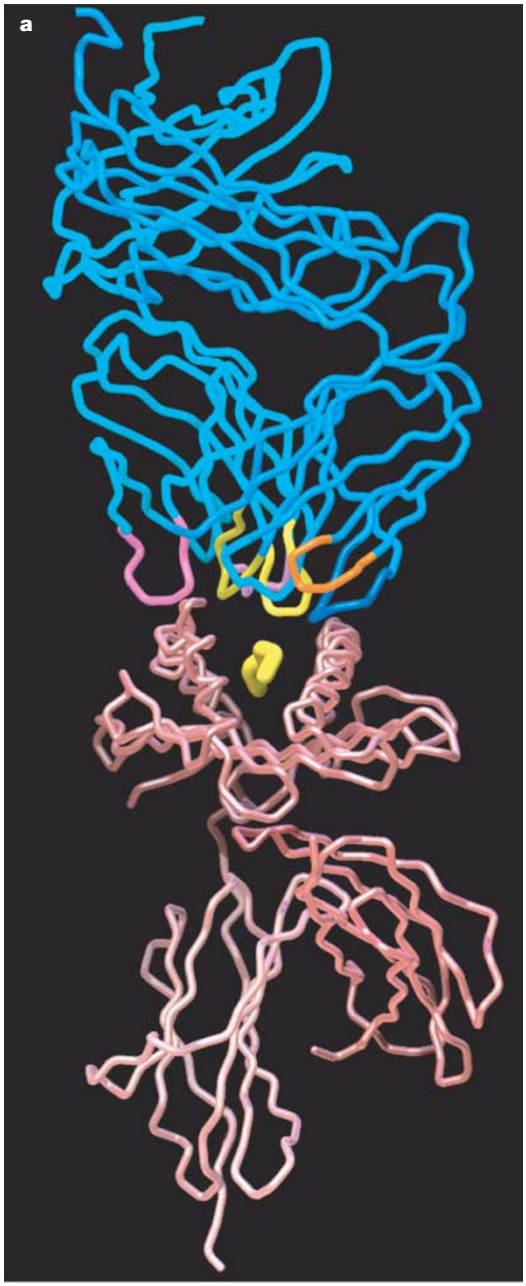

Figure 1 | **Overview of the interaction between T-cell receptor (TCR) and peptide–MHC (pMHC). a** | Side view of the TCR–pMHC interaction (the TCR is shown in blue; different complementarity-determining region (CDR) loops are highlighted; the MHC molecule is shown in red and its bound peptide in yellow). **b** | Top view of the pMHC complex, with the CDR loops of the TCR superimposed. (The MHC molecule is green, the peptide is yellow, the CDR loops of the TCR α-chain and β-chain are pink and blue, respectively.) Structures represent the 2C–dEV-8 complex, kindly contributed by K. C. Garcia (Stanford University).

TCR structure; and second, FUNCTIONAL DIVERSITY, arising from differential maturation/differentiation towards different effector functions, even in cells expressing the same TCR (FIG. 2).

Factors influencing the T-cell repertoire

Quantifying GOD: recombinatorial diversity. The puzzle of generation of immune-receptor diversity (GOD) literally enjoyed deity-like status among immunologists until its molecular elucidation[12]. TCR-αβ diversification mainly occurs in the thymus by stochastic V(D)J recombination of non-contiguous gene segments. Diversity is further enhanced by imprecise joining of nicked segments, by addition of non-germline nucleotides by DNA-repair machinery and by pairing of different TCR-α and -β segments. The theoretical estimate of the diversity that is generated by such a system is enormous[13]: more than 1×10^{15} TCR-αβ receptors could be formed (FIG. 2). Moreover, some T cells will express two TCRs (two different TCR-α chains pairing with the same TCR-β chain, due to poor TCR-α allelic exclusion). The upper limit of incidence of such cells would be 30%[14–16], however, most of the 'second' TCRs in such cells will be unlikely to function properly with self-MHC molecules, and their contribution to diversity is questionable at present. Both positive and negative intrathymic selection immediately and markedly limit the diversity of the generated repertoire, and estimates of this reduction range from 3 to 100 fold[17–20]. So, the theoretical upper limit of diversity of the peripheral TCR repertoire would be more than 1×10^{13}. A mouse contains ~$1–2 \times 10^8$ T cells in total[21]; and humans have up to 1×10^{12} T cells[22]. It is known that at least some T cells express the same TCR — that is, an individual TCR is not necessarily confined to one cell, due to intrathymic and post-thymic homeostatic expansion of naive T-cell populations, and homeostatic and antigen-induced expansion of memory T-cell populations. There cannot be more TCR specificities than total T cells at any one given moment in the body, so although theoretically there could be 1×10^{13} TCRs, only ~1×10^8 can be present at one time in any given mouse.

Estimates of the actual peripheral TCR-αβ diversity were recently obtained by extrapolation from molecular measurements of TCR diversity using the immunoscope technique[23,24]. These calculations (BOX 1) estimate the actual TCR-αβ diversity of naive human T cells at 2.5×10^7, and that of memory cells at 100-fold less[22].

FUNCTIONAL DIVERSITY
The ability of activated T cells, all specific for a single epitope, to have one or more distinct effector functions, such as proliferation, cytokine secretion (different cytokines), cytolysis, migration and homing.

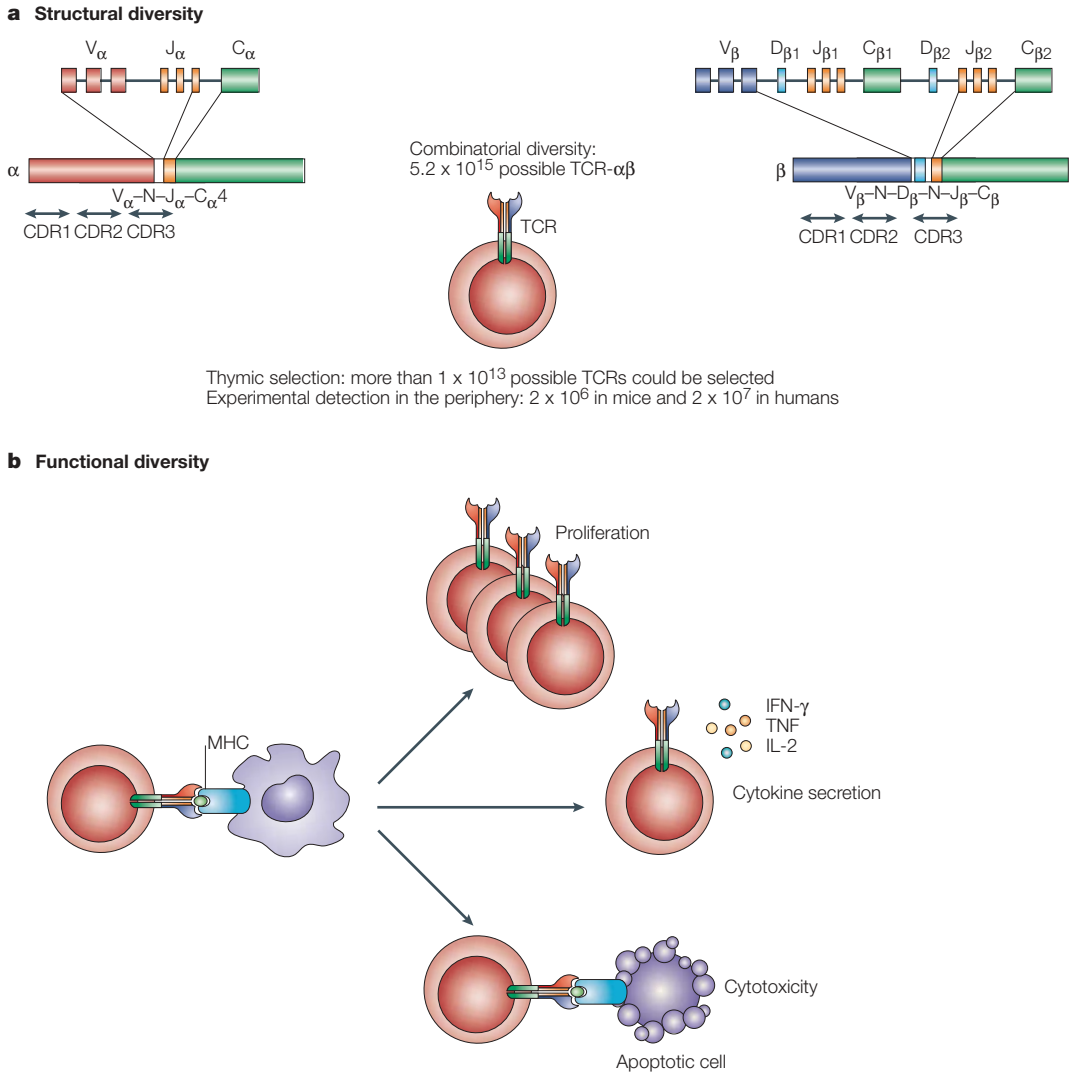

Figure 2 | **T-cell diversity. a** | Structural diversity of the T-cell receptor (TCR). Functional TCR-αβ chains are generated through the recombination of a variable (V), joining (J) and diversity (D) segment to a constant region (C) in the case of the β-chain and V-J-C in the case of the α-chain. Recombinatorial diversity is further enhanced by addition and deletion of nucleotides (N) at the junctions between the segments, but is reduced by thymic selection. **b** | Functional diversity of antigen-specific T cells. Following antigen exposure, activated T cells have one or more distinct effector functions: proliferation, cytokine secretion (different cytokines) and cytolysis, as well as a differential propensity for migration and homing, for example. CDR, complementarity-determining region; IFN-γ, interferon-γ; IL-2, interleukin-2; TNF, tumour-necrosis factor.

The mouse TCR-αβ repertoire was estimated at 2×10^6 different TCRs[25]. Both figures are thought to be minimal estimates, but given the limits in the number of precursors and total T-cell numbers, they convey realistic, standard values. If so, humans would have only about tenfold higher TCR-αβ diversity than mice; but the size of each clone expressing the same TCR would differ by 20–100 fold, with ~1,000–4,000 and ~20–50 cells/clone, in humans and mice, respectively[22,25].

The potential/theoretically achievable repertoire is therefore many orders of magnitude larger than the one that can be expressed in any given individual at any given moment. This enormous reserve should lead to differences in expressed TCR repertoires even between genetically identical organisms. Indeed, such differences were observed in analysis of naive T cells in individual mice of the same inbred strain that have non-overlapping TCR repertoires — only about 20–25% of TCR-β sequences were shared[26]. In another study, several alymphoid animals received spleen cells from the same donor[27] and when recipient TCR repertoires were analysed, up to 80% of TCR sequences were unique. Similarly, genetically identical mice mount unique responses to lymphocytic choriomeningitis virus (LCMV), with different TCR sequences[28]. So, there is rather high diversity, but a limited number of copies of each clone in the body; much of the potential repertoire can and will be used, but limits imposed by total T-cell numbers allow only a fraction of the potential repertoire to be expressed in each individual at any given moment. Even with these limitations, there is still very high ($1 \times 10^{6-7}$) diversity to the system.

Box 1 | **Experimental estimates of T-cell receptor (TCR) diversity**

- First, the complementarity-determining regions 3 (CDR3) of a given Vβ–Jβ rearrangement (for example, Vβ10–Jβ1S2, 0.3% of all T cells) in a T-cell population are amplified by PCR after reverse transcription of RNA (RT-PCR); products migrate on a gel by CDR3 length (CDR3 length polymorphism analysis[23,24]). All products with CDR3 length of N nucleotides migrate as one band; typically 6–8 bands are visible for one Vβ–Jβ rearrangement — arranged in a Gaussian profile[22–25].
- Second, relative abundance of each of the bands is quantified; one band (for example, 30 nucleotides (corresponding to 10 amino acids), containing, for example 11% of all amplified sequences) is excised.
- Third, the RT-PCR products belonging to that band are exhaustively sequenced, until no new sequences appear. This number of sequences (X) is the diversity of T-cell receptor (TCR) Vβ sequences belonging to the Vβ10–Jβ1S2 combination with a CDR3 of 10 amino acids in length.
- Fourth, extrapolate to all TCR Vβ sequences belonging to the Vβ10–Jβ1S2 combination, regardless of CDR3 length; (here, Y = 11%, so, $X \times 0.11$); extrapolate that number to all Vβ–Jβ combinations (Vβ10–Jβ1S2 is 0.1% of total combination). This is total β-chain diversity. The number of α-chains found to pair with a given β-chain in a T-cell population is used to calculate total diversity.
- Numbers: the mouse repertoire is estimated at 2×10^6 and the human repertoire at 2.5×10^7 different TCR clonotypes[22,25].

Effects of TCR–pMHC interactions on T-cell diversity. Whereas the genomic organization of the TCR locus and the biochemistry of TCR-gene rearrangement allow the generation of a broad TCR repertoire, interactions of the formed repertoire with self-peptide–MHC complexes decisively shape its scope and reactivity. What is the relationship between recombinatorial diversity and its constriction due to MHC selection? Surprisingly, nearly identical estimates of TCR-β diversity were obtained in mice expressing five MHC class I/II alleles and those expressing three[25], indicating an intriguing, but speculative possibility, that there might be a predetermined, optimal level of TCR diversity irrespective of MHC contacts. However, TCR–pMHC interactions during development will cause losses or gains of certain TCRs and will thereby shape TCR STRUCTURAL AVIDITY (FIG. 3). These interactions can also modulate the density of cell-surface receptors (TCRs, co-receptors and adhesion molecules) as well as the efficacy/sensitivity of TCR/co-receptor signalling (T-cell FUNCTIONAL AVIDITY), by still poorly understood mechanisms (FIG. 3).

MHC molecules are themselves markedly polymorphic, and each of them preferentially selects some and deletes other TCR specificities, creating unique 'holes' in the TCR repertoire. Even focused changes in pMHC complexes can lead to differences in the expressed, functional TCR-αβ repertoire, and these differences can be mapped to both positive and negative selection[29,30]. For example, a change in only a few functionally important amino acids on the floor of the H–$2K^b$ peptide-binding site produces a new allele, H–$2K^{bm8}$, which results in increased positive selection of herpes simplex virus (HSV) glycoprotein $B_{498–505}$ (gB-8p)-specific T cells in bm8 mice compared with B6 mice and a complete lack of $ovalbumin_{257–264}$ (OVA-8p)-specific T cells in bm8 mice, but not B6 mice[31–33]. This markedly impacts HSV1 resistance[32], such that bm8 mice can withstand a high HSV1 dose. Conversely, TCR repertoire purging by negative selection of autoaggressive T-cell populations reduces T-cell diversity[34], which can produce holes in the T-cell repertoire (the inability to respond to certain antigens) or, alternatively, selection of TCRs of lower structural avidity. This latter phenomenon was shown by lower binding of pMHC tetramer reagents — a phenomenon seen in normal[35] or TCR-β-transgenic animals in the context of an intact TCR Vα-chain repertoire[20].

Besides producing a hole in the repertoire, negative selection can have additional biological consequences. A recent study examined T cells in a transgenic mouse model in which the nucleoprotein (NP) of LCMV is expressed by the islet cells of the pancreas and the thymus[35]. These mice do not show any signs of autoimmunity unless infected with LCMV, in which case the animals mount an antiviral T-cell response that results in a state of islet-specific autoimmunity. This study found that the $CD8^+$ T-cell response to the immunodominant NP T-cell epitope (NP118) was of low structural (as determined by pMHC-tetramer binding) and functional avidity[8]. Not only were the low-avidity NP118-specific T cells less capable of responding to NP118 peptide, but they also responded poorly to altered peptide ligands. So, thymic purging not only removes T cells of the highest structural avidity, but also removes the most promiscuous T-cell populations that can respond to similar (although not identical) peptides[35]. This preferential deletion of the most promiscuous/crossreactive T cells might explain why many individuals have autoreactive T cells but relatively few suffer from autoimmune diseases. Indeed, in one case, high avidity for MHC predicated high crossreactivity[36]. Interestingly, analysis of the low-avidity NP118-specific Vβ T-cell repertoire used in the NP-transgenic mice showed similar Vβ usage to non-transgenic mice capable of mounting high-avidity LCMV-specific T-cell responses[37]. This indicates that the range and/or frequency of low-avidity peptide-specific T-cell subsets within the immunodominant Vβ family surpasses that which might be found in other Vβ families. Similar results were found in male mice transgenic for the TCR-β chain specific for the male antigen (H-Y) — T cells of low structural avidity used the same Vα chain, Vα9, as T cells of high structural avidity observed in female mice in which no negative selection occurred[20]. In this case, the preferred Vα9 CDR3 sequences with an optimal length of amino acids were deleted in the low-avidity T-cell populations, but notably, other Vα chains were still not used.

Together with structural and functional data that show preferential TCR V-domain interactions with pMHC[38–41], these data indicate that germline-encoded structural features both inside and outside of the vital CDR3 region might be crucial in TCR interactions with certain pMHC molecules, collectively providing evidence that at least some cases of TCR IMMUNODOMINANCE might be determined at the level of TCR structure, as opposed to TCR/T-cell frequency.

STRUCTURAL AVIDITY
Is determined by the direct binding affinities of multiple cell-bound T-cell receptor molecules for peptide–MHC (pMHC); most commonly measured by staining with pMHC multimers.

FUNCTIONAL AVIDITY
Relates the binding avidity of an antigen-specific T cell to a measurable biological function (proliferation, cytokine production or cytolytic activity) at different doses of peptide antigen.

TCR IMMUNODOMINANCE
The preferential recruitment of a T-cell population that expresses restricted T-cell receptor (TCR) elements (Vα, Vβ) in response to a given epitope, mainly determined by the structural characteristics and avidity of the responding T-cell population.

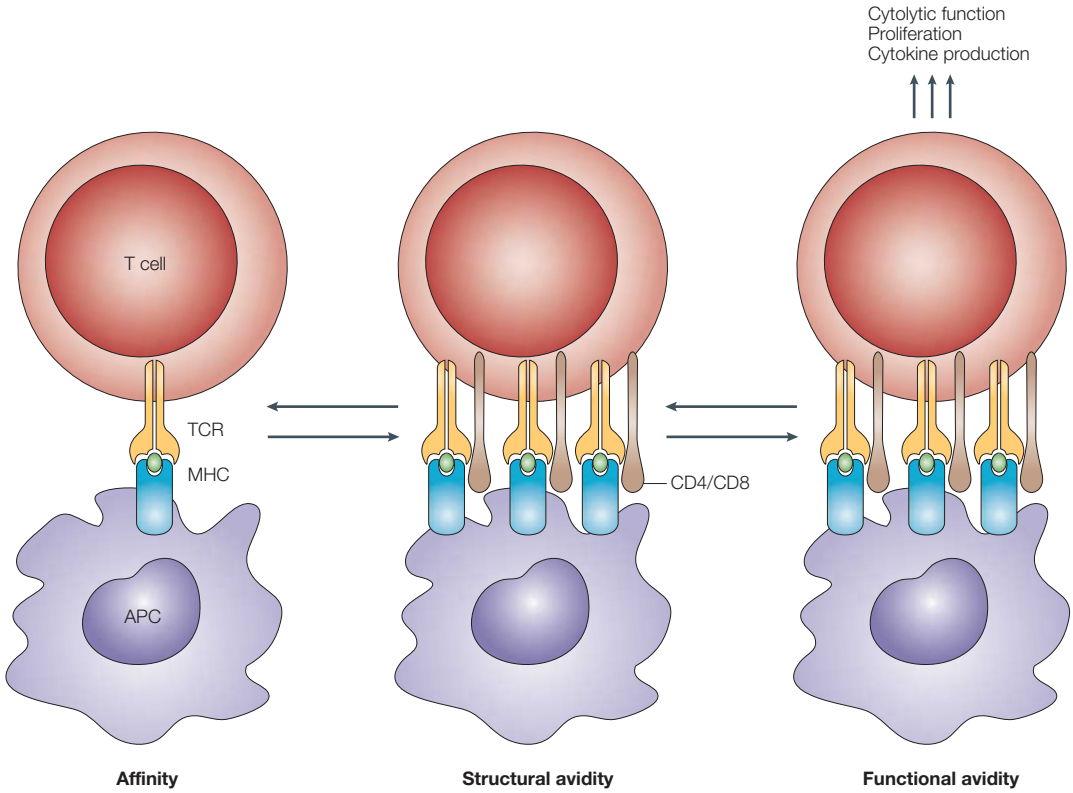

Figure 3 | **Overview of the concepts of affinity and avidity.** Affinity refers to the steady-state association constant between a monovalent receptor and its ligand, in this case a single T-cell receptor (TCR) and peptide–MHC (pMHC) complex. Structural avidity is the steady-state association constant between multiple cell-bound receptors and ligands and is determined by the direct binding affinities of multiple TCRs to their pMHC complexes. Functional avidity depends on the relative kinetics of signalling that translate into measurable biological functions such as proliferation, cytokine production or cytolytic function. APC, antigen-presenting cell.

Negative selection could also theoretically promote some structural and functional diversification through receptor editing, whereby serial TCR-α rearrangements are induced by negatively selecting ligands[42,43], replacing the original TCR-α chain with other chains. So, instead of one specificity that would have been negatively selected, several could be generated by receptor editing. These effects have been observed in some TCR-transgenic models[42,43], but not in others[44], and, therefore, the physiological significance of this mechanism remains incompletely understood.

The examples mentioned illustrate instances of structural changes to the TCR repertoire induced by positive or negative selection. Moreover, there are many examples in which TCR contact with self-peptide–MHC results in the functional modification of T-cell reactivity. These include downregulation of important cell-surface molecules such as TCRs, CD4 or CD8 (REFS 45,46) or functional anergy, all of which have been observed in TCR-transgenic systems.

With the TCR repertoire being set by these forces, T cells that have left the thymus are now charged with the crucially important task: protection against pathogens. At this point, functional diversity after contact with antigen is established[3–6]. It is still unclear to what extent TCR structure and avidity can determine functional diversification in and outside of the thymus, and to what extent functional features are programmed by TCR-independent events. The one feature that is influenced by TCR avidity (and thereby indirectly by diversity) seems to be the skewing of T helper 1 (T_H1)/T_H2-cell responses, where higher avidity and longer TCR–pMHC contacts have been linked to T_H1-cell function[47]. It will be of interest to extend such studies to other functions, including migration, proliferation and secretion of other cytokines.

Muddying the waters: TCR crossreactivity. An important factor at the interface of structural and functional diversity is TCR crossreactivity — the ability of a TCR to recognize several different peptides bound to self-MHC molecules (BOX 2). (TCR crossreactivity on non-self MHC molecules is outside the scope of this review). One problem facing the immune system is how to use a finite TCR repertoire, distributed on a finite number of T cells, to deal with a very large number of epitopes. It is essential for immune defence that sufficient numbers of cells are deployed to combat infection. Simultaneously, specificity of recognition must be high enough for the repertoire to respond to foreign, but not to self, peptides. These conditions could be met by a large, diverse and highly selective

Box 2 | **T-cell receptor crossreactivity**

Parameters

- How many peptide–MHC (pMHC) complexes are there?[49] The complexity of 11-mer peptides bound to a MHC class II molecule (I-E^k) is 6×10^{12} (assuming that 3 out of 11 positions are anchor residues and can tolerate 4, 10 and 7 amino acids, respectively; all other residues vary freely with all 20 amino acids allowed). The complexity of 9-mer–MHC class I complexes is 1.3×10^{10}.
- How crossreactive are T cells?[49] Each T cell would optimally recognize 1×10^6 to $>1 \times 10^8$ ligands.

Issues

- How many pMHC complexes are really out there? Based on the parameters above, ~6.5×10^8 8-mer–MHC class I complexes should exist for a given allele. Yet, experimentally, H–$2K^b$ imposes numerous restrictions on the 8-mers it binds in a stable and immunologically relevant manner (no Asp, Glu, His, Arg, Trp, Phe, Tyr or Lys at position 2 (P2); no His, Arg, Lys or Trp at P3; only Phe and Tyr at P5; only Ile, Leu, Val and Met and Ala at P8 (REFS 90–94) (J.N.-Ž. *et al.*, unpublished observations). Other negative influences: consecutive amino acids with negative or positive charges; two prolines anywhere in the molecule; two cysteines spaced by one or more amino acids; small residues at both P2 and P3 (Gly or Ala). Thereby, H–$2K^b$ probably really binds $0.5–1 \times 10^6$ chemically different peptides, consistent with peptide-elution data[95]. Similar restrictions exist in pMHC class II complexes[96]. Further, as MHC class-II-binding peptide residues that extend beyond the core of 9 amino acids are rarely directly recognized by T-cell receptor (TCR)[97], such flanking residues often do not influence TCR recognition. So, accounting for the variability of flanking residues is likely to further overestimate the number of pMHC class II ligands (as there are 3.2×10^6 different 14-mer peptides that share an identical 9-mer core and differ only at flanking residues).
- What is the true TCR promiscuity? TCR promiscuity is defined as the ability to react to different pMHC surfaces. In both MHC class I and class II complexes with peptides, only 3–4 peptide amino acids are exposed to and contacted by the TCR[7,8,97]. Although certain variations at buried, MHC-contacting residues can indirectly affect TCR recognition, many more do not. So, recognizing variant pMHC complexes with an identical TCR-contact surface is not TCR promiscuity (crossreactivity).
- Crossreactivity is usually measured at unphysiologically high doses of peptide; such crossreactivity is typically devoid of *in vivo* protective capability[81,82].
- It follows that the TCR capacity to recognize disparate pMHC surfaces, although real and important, was both structurally and biologically overestimated in REFS 49–51.

TCR repertoire; alternatively, they could be met by a combination of diversity and promiscuity — by crossreactive recognition of multiple peptides. Indeed, there is ample evidence for both a high degree of specificity among TCRs, and for considerable TCR crossreactivity[48–50]. So, assuming that a mouse has ~ 2×10^6 different αβ-TCRs[25], it is important to calculate how many different peptides this repertoire could be confronted with. Using the maximal number of combinations for an 11-mer peptide bound to a MHC class II molecule, and minimal restriction on the type of amino acids that are allowed at different positions, Mason[49] calculated the number of antigenic peptides that can potentially be presented by one MHC class II molecule to be ~6×10^{12} (BOX 2). He further estimated that each TCR should be able to interact with ~1×10^6 peptides for optimal reactivity, as a compromise between the advantage of having a high frequency of T cells that respond to a given epitope and the disadvantage of a high level of intrathymic clonal deletion. Another study found that a self-reactive MHC class II-restricted T-cell clone could recognize three peptides from a synthetic peptide library containing 8×10^6 HLA-DR3-specfic peptides[51]. By calculating the possible combinations of 14-mer peptides in nature, the authors came up with an estimate that was similar to Mason's, that one TCR would recognize $>1 \times 10^6$ peptides. If these estimates are correct, crossreactivity would contribute to diversity of antigen recognition almost to the same extent as structural TCR-αβ diversity. Moreover, if broad crossreactivity is functionally relevant for pathogen defence, as suggested by these authors, crossreactivity should be able to overcome many situations in which there are restrictions in structural diversity. However, although there is support for the idea that some crossreactivity might alter the memory T-cell repertoire[52], the extent of crossreactivity and its limits in immune defence are still poorly understood.

IMMUNODOMINANT EPITOPES One or a few epitopes that a pathogen-specific T-cell response focuses on. Immunodominance is mainly determined by antigen processing and presentation, the abundance of the antigenic protein, the ability of the epitope to bind the given MHC molecule and the availability of a responding T-cell population.

T-cell diversity in action: enter the pathogens

The main task of optimally calibrated diversity is to ensure efficacious reaction to pathogens while avoiding self-reactivity. To that effect, important questions related to immune defence are: by which mechanism does diversity contribute to pathogen-specific defence? How much diversity is required for successful defence against pathogens? and how many copies of the same T cell is optimal for protection?

Conceptually, TCR structural diversity is important on at least two levels: first, providing optimal structural avidity to respond efficiently to IMMUNODOMINANT EPITOPES; and second, to prevent pathogen escape by mutation. The two facets, although complementary in aims, deal with different tasks. To achieve the first, the best and most efficient TCRs to target a single epitope must be selected from the available diversity[32]; if the pathogen mutates infrequently, and if the response is successful, the mission is accomplished. For mutating pathogens (for example, HIV, influenza virus and hepatitis C virus, HCV), however, it is probable that diversity also has to provide the 'recognition reserve' to target mutated epitopes or secondary epitopes if the primary ones 'escape'. Failures of T-cell immunity in such cases might signal that diversity (and crossreactivity) can no longer keep pace with such pathogens. Moreover, it is probable that several T-cell functions need to be carried out, perhaps in a correct temporal order, to limit and eliminate the pathogen. In which case, the diversity of T-cell effector functions would be important. Such diversity was observed when populations of antigen-specific T cells (as judged by binding of specific pMHC tetramers) were found to have heterogeneous proliferation, cytotoxic T lymphocyte (CTL) activity, cytokine and chemokine secretion, and expression of natural killer (NK)-cell receptors, chemokine receptors and integrins, as well as migratory patterns and other features[3–6,53–56]. Similar functional heterogeneity was also observed in T cells expressing the same TCR, indicating that at least part of functional heterogeneity could be independent of TCR structural features[5,53,54,57,58]. However, it remains unclear whether and how structural TCR diversity influences functional diversity, and what is the impact of functional diversity on pathogen resistance.

Taking the measured and extrapolated TCR-αβ diversity numbers[22,25] at face value, one is compelled to conclude that this diversity (~2.5×10^7 in humans and ~2×10^6 in mice) apparently confers good survival fitness to both mice and humans. When Mason's values of TCR crossreactivity in mice[49] are superimposed over these numbers, it follows that the TCR repertoire should recognize between $2 \times 10^{6-7}$ (if all of the TCRs in an organism crossreact with the same set of antigens) and $2 \times 10^{12-13}$ pMHC complexes (if each TCR cross-reacts with a unique set of ~$1 \times 10^{6-7}$ antigens). As neither of these two extremes seems probable, the relevant number is somewhere in between. This numerical window is discussed later.

There are several difficulties in assessing the role of TCR diversity in pathogen resistance. To observe an effect of TCR-repertoire restriction, T cells need to be the main determinants of pathogen resistance, and the host has to dominantly react to one or, at the most, a few strongly immunodominant pathogen epitopes. It is easier to observe such effects in inbred laboratory animal models, as the outbred nature of human and non-human primate populations further decreases the chance of decisive IMMUNODOMINANCE. Nevertheless, two physiological and/or experimental situations provide important clues to this difficult question. First, numerous pathogens, including HIV, simian immunodeficiency virus (SIV) and HCV, mutate under the pressure of an ongoing immune response, as indicated by the isolation of escape variants of pathogen epitopes[59–64]. These variants typically are altered so as to prevent MHC binding, TCR binding or both. Isolation of TCR-contact mutants is evidence for the failure of TCR diversity, particularly if this results in a lack of pathogen control and increased morbidity and mortality. Indeed, this means that the pathogen, by generating one or more variants of its epitopes, has managed to exhaust the diversity of TCRs that are available to contain it. The second, more direct, line of evidence comes from analysing T-cell reactivity in cases of natural or experimental restrictions of TCR diversity. Almost invariably, such studies show reduced reactivity to several antigens, and in some cases, marked holes in the T-cell repertoire were observed (see later), even if connections to pathogen resistance were infrequently examined (TABLE 1).

A case in which TCR diversity might not influence pathogen resistance. Targeted disruption of the enzyme terminal deoxyribonucleotidyl transferase (TdT) leads to a ten-fold reduction of TCR diversity[65]. TdT is essential for efficacious addition of non-templated nucleotides to the CDR3 regions in the course of V(D)J recombination, and consequently Tdt-deficient animals show impaired CDR3 diversification[66]. When challenged with LCMV or Sendai virus, the animals were capable of withstanding infection[65]. These mice also had a high degree of TCR crossreactivity to other antigens[67]. Therefore, it can be speculated that these mice might compensate for reduced structural TCR diversity by increased crossreactivity. However, it is not known whether this might result in increased autoimmunity.

Reduced TCR diversity impairs the immune response. Experimental introduction of TCR transgenes is known to reduce the diversity of the TCR repertoire. In particular, introduction of a TCR-β transgene into the germline essentially excludes expression of endogenous TCR-β, thereby reducing diversity to that provided by TCR-α alone (estimated to be ~4×10^5 in mice[25]). In one such model, transgenic mice that express the β-chain specific for OVA-8p presented by H–$2K^b$ (OT-1β-transgenic mice) were unable to reject F1 bone marrow, revealing a hole in the TCR repertoire[68].

Natural reductions of the TCR repertoire have yielded even more informative data. A spontaneous reversion of the X-linked immunodeficiency (Xid) mutation was studied in one patient[69]. A single progenitor cell was affected by this reversion, and it managed to generate at least 2.5×10^4 distinct TCRs. This repertoire seemed to protect from major infections early in life, but the immune response to mitogens, polyclonal activators and two antigens was 7–100-fold reduced compared with controls.

Parts of the TCR locus have been found to be deleted in both inbred laboratory and outbred, wild-dwelling rodents. A deletion in New Zealand white (NZW) mice affects Cβ1, Dβ2 and all of the six Jβ2 segments and theoretically leads to a threefold reduction in TCR-αβ diversity. Woodland *et al.*[70] tested the ability of T cells from these animals to respond to 22 antigens and found that responses to 11 out of 22 were markedly reduced, perhaps indicating altered functional avidity. Another genomic deletion affects the TCR Vβ locus in mice of the tcr^a haplotype (both wild-type and laboratory mice, such as C57L mice) , knocking out TCR Vβ5, 8, 9, 11, 12 and 13, and replacing amino acids in others (Vβ10), results in a net twofold reduction (~50%) in diversity. Notably, these mice were unable to respond to two MHC class-II-restricted determinants, sperm-whale myoglobin and myelin basic protein, indicating classic holes in the TCR repertoire[71].

Informative restrictions in T-cell responsiveness were also found in wild-type settings. The $CD8^+$ T-cell response of C57BL/6 mice to the immunodominant HSV gB-8p epitope is dominated by TCRs containing Vβ10 (up to 60–65%) and Vβ8 (15–25%)[72,73]. If the gB-8p determinant is deleted by engineering a mutant virus[74], the response to the virus is reduced by 70%, indicating the ability of T cells to compensate by recognizing other viral epitopes is poor. Moreover, if the mice express the tcr^a haplotype, which prevents TCR Vβ10 usage, the response to the virus is reduced by 60%[75], indicating that no other TCR Vβ can compensate for the loss of immunodominant interactions between Vβ10 and gB-8p. The basis of this immunodominant interaction seems to be structural: preferential usage of Vβ10 is strictly dependent on the identity of TCR-exposed peptide residues[76], and on TCR amino-acid residues encoded by the germline Dβ1 segment[40] — alterations in either one eliminated Vβ10 participation in the response and reduced the response by >50%.

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A phenomenon that arises from T-cell economy in response to antigen. Out of all possible combinations, only a few T cells will respond to a few epitopes of the pathogen, so as to produce focused, effective responses.

Table 1 | **Impact of TCR diversity reduction on immunity**

Model	Reduction in diversity	Impact on repertoire	References
Tdt-knockout mice	90%	Resistant to LCMV and Sendai virus	65
OT-1β-transgenic mice	4 × 10^5 TCRs (98% reduction)	Failure to reject F1 bone marrow	68
Spontaneous reversion of Xid in human patient	2.5 x 10^4 TCRs (99.9% reduction)	7–100-fold reduction in responses to mitogens and antigens	69
NZW mice: deletion of Cβ1, Dβ2 and six Jβ2 segments	60% reduction in TCR diversity	Severe reduction in responses to 11/22 antigens	70
C57L mice: deletion of TCR Vβ5, Vβ8–Vβ13	50% reduction in TCR diversity	No response to HSV1 (MHC class I restricted response), sperm-whale myoglobin or myelin basic protein (MHC class II restricted response)	40,71
The 'limited' TCR mouse (Vβ transgenic + one Vα and two Jα segments)	Not determined, but should be >10,000 times reduced	Preferrential positive selection. Antigen- or pathogen-specific responses not reported	98

HSV1, herpes simplex virus 1; LCMV, lymphocytic choriomeningitis virus; NZW, New Zealand white mouse strain; OT-1β, TCR-β-chain specific for an ovalbumin-derived peptide presented by H–$2K^b$; TCR, T-cell receptor; Tdt, terminal deoxynucleotidyl transferase; Xid, X-linked immune deficiency.

In the context of pathogen resistance, it is important to note that even subtle differences in TCR diversity can affect pathogen resistance. As mentioned, the MHC class I co-isogenic mouse strains B6 and B6.C-H–2^{bm8} (bm8) differ in diversity of the TCRs specific for the HSV1-derived peptide gB-8p[31,33], with bm8 mice exhibiting higher diversity at the level of both Vβ usage and CDR3 length. This relatively subtle gain in the diversity of the TCR repertoire specific for a single viral epitope was shown to translate into higher resistance to this pathogen[32]. One of the ways in which diversity helped pathogen resistance was by generating a diverse pool from which to recruit high-avidity T cells that eliminate pathogen-infected cells with exceptional efficacy: B6 mice, that showed low diversity, could only mobilize less efficient T cells of markedly lower avidity. Other data from that study indicated that diversity might contribute to pathogen clearance by additional, presently unidentified, mechanisms not involving avidity[32].

Aging, HIV and bone-marrow transplantaion. One physiological (aging) and several pathological/therapeutic (HIV infection/highly active antiretroviral therapy (HAART), T-cell leukaemia and bone-marrow transplantation) conditions yield reduced TCR diversity. Phenomenology of these conditions is often similar — T-cell clones, invariably of the $CD8^+$ T-cell phenotype, tend to expand, forming T-cell clonal expansions (TCEs) that take over the space and thereby reduce diversity of the remainder of the T-cell pool[77–79]. Our recent results (Messaoudi *et al.*, unpublished observations) indicate that such TCEs initially result in focused holes in the repertoire, mostly impairing responses that require those TCR families to which the TCE belongs. Only much later, in the most extreme cases, might this lead to wider defects in antigen recognition. Such naturally occurring states represent potentially informative models to dissect further the relationship between TCR diversity and T-cell-precursor frequency in pathogen resistance and to investigate that mechanisms that regulate peripheral maintenance of T-cell diversity.

Diversity, crossreactivity and pathogen-specific T-cell numbers. Results from Tdt-deficient mice[80], and perhaps to some extent, from the Xid-revertant patient[69], indicate that TCR crossreactivity might be able to compensate for reduced structural diversity. However, other studies[32,40,68,70,76,81] highlight the limitations of that potential — relatively small reductions in TCR diversity can readily produce holes in the TCR repertoire (TABLE 1). If TCR crossreactivity is as high as has been proposed[49], it is difficult to imagine how a mere 50% reduction in functional diversity would result in such clear defects of reactivity. It is even more difficult to explain lack of such compensation in the study of Messaoudi *et al.*[32], in which there is an even smaller reduction in diversity. This abundance of cases in which crossreactivity seems to be incapable of compensating for inadequate structural TCR diversity[32,40,68,70,76,81] indicates that either functional crossreactivity is smaller *in vivo* than *in vitro* or more limited to structurally related antigens, making it less effective at a global level. Supporting this view, crossreactivity detected *in vitro* using high-peptide doses was shown to be devoid of protective activity *in vivo*[48,82]. This is consistent with what we believe is an overestimation of TCR crossreactivity in recent literature[49–51] (BOX 2).

An alternative explanation to the discrepancy between estimated high crossreactivity and its inability to compensate for antigen recognition is that we still do not understand the relationship between structural TCR diversity and crossreactivity. Indeed, full understanding of this relationship will require systematic and simultaneous TCR and pMHC site-directed mutagenesis experiments, as well as new and incisive quantitative studies of pathogen resistance in these and other relevant models.

As mentioned, the large diversity of available TCRs, the structural avidity of those TCRs and the probable need for those T cells that express them to have the optimal combination of effector functions are all required to combat pathogens. Another requirement is that precursor frequencies of such T cells must be sufficiently high for pathogens to be eliminated[83–85]. So, what is the winning combination? From the studies of a group

from the Pasteur Institute, Paris, France[22,25], we know that the main difference between mice and humans is not diversity (~10 times higher in humans), but rather the number of T cells that express a given TCR (nearly 100 times higher in humans). It was hypothesized that this difference might be determined by the area (size) of the organism that needs to be patrolled[25], reminiscent of the proposed 'protection' size of Cohn and Langman[86]. This hypothesis is readily testable — one would expect that clonal redundancy in rats and primates would fall between that of mice and humans. If so, the minimal frequencies will be determined by the size of the area that each clone has to patrol and the number of naive clones specific for pathogen epitopes.

Numbers of naive CTL precursors were recently estimated at 100–200 by two groups investigating two different responses — LCMV-derived gp33 peptide bound to H–2D^b (LCMV gp33/H–2D^b)[87] and HLA-A2-derived peptide bound to H–2K^d (REF. 26) — and the diversity estimates indicated that 20 or fewer clones made up this number of precursors[26,87]. But a third group revisited the LCMV gp33/H–2D^b response as well as studying the response to two other antigens, and concluded that a log series mathematical model fits all of the data[88] (S. Perlman, personal communication). Estimates using this model indicate that at least 500–1,500 clonotypes are present in the naive repertoire. The two sets of numbers would implicate markedly different crossreactivity levels: assuming that there are 1×10^7 different pMHC complexes that need to be recognized (BOX 2), a single TCR would have to crossreact with between 100 (REFS 26,87) and 10,000 (REF. 88) different pMHC molecules. Although these discrepancies clearly need to be resolved, an interesting complementary estimate was made in the LCMV gp33/H–2D^b model. It was shown that 5×10^7 virus-specific CD8$^+$ T cells/m^2 body surface area and 50-fold fewer CD4$^+$ T cells might be enough for viral clearance[89]. Similar estimates were made in other systems[83]. In this regard, it will be interesting to test whether there are upper limits of virus-specific cells, beyond which no additional protection is gained against pathogen attack.

Future challenges

In conclusion, important initial steps have now been made in quantifying TCR diversity in different lymphoid compartments and across various biological processes, providing an excellent platform from which to continue enquiries into the role of TCR diversity in immune defence. Despite such encouraging advances, the topic to be tackled is among the most challenging in modern immunology. There are several remaining questions: which aspects of TCR diversity are the most important in immune defence (for example, TCR diversity could function as a 'supermarket' from which one might need one or more of the following: best avidity, best functional effector match, best combination of effector functions, best prevention of escape)? What is the functional relationship between structural TCR diversity and cross-reactivity? How useful is TCR crossreactivity in immune defence against pathogens? Can structural diversity be restored and how? Can we modify functional diversity and/or avidity?

Despite the technically challenging nature of these questions, the rewards associated with their successful resolution are considerable and include rational vaccine design to elicit the desired end-products of TCR diversity (T cells of predetermined TCR diversity/avidity and/or effector function) and the restoration and manipulation of TCR-repertoire diversity in cases of its natural or pathological loss. One can certainly look forward with excitement to the journey towards these goals.

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Competing interests statement

The authors declare that they have no competing financial interests.

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