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The authors declare no competing financial interests.

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OPINION

Why must T cells be cross-reactive?

Andrew K. Sewell

Abstract | Clonal selection theory proposed that individual T cells are specific for a single peptide–MHC antigen. However, the repertoire of αβ T cell receptors (TCRs) is dwarfed by the vast array of potential foreign peptide–MHC complexes, and a comprehensive system requires each T cell to recognize numerous peptides and thus be cross-reactive. This compromise on specificity has profound implications because the chance of any natural peptide–MHC ligand being an optimal fit for its cognate TCR is small, as there will almost always be more-potent agonists. Furthermore, any TCR raised against a specific peptide–MHC complex *in vivo* can only be the best available solution from the naive T cell pool and is unlikely to be the best possible solution from the substantially greater number of TCRs that could theoretically be produced. This 'systems view' of TCR recognition provides a plausible cause for autoimmune disease and substantial scope for multiple therapeutic interventions.

T cells recognize peptides bound to MHC class I and class II molecules at the cell surface[1]. The specificity of this recognition is conferred by the clonotypic αβ T cell receptor (TCR), which is made from two separate chains manufactured from variable (V), diversity (D), joining (J) and constant (C) gene fragments through a process of somatic gene rearrangement. This process involves nucleotide insertions and deletions at V(D)J junctions in each chain. The 'randomization' of V(D)J junctions and the fact that the TCR is a heterodimer of two separately rearranged chains results in a theoretical repertoire of $>10^{15}$ unique αβ TCRs in the mouse[2,3]. The theoretical number of possible TCRs in humans is likely to be orders of magnitude larger, as humans possess 54 TCRβ variable genes as compared with the 35 genes in mice, with all other variables being comparable[4].

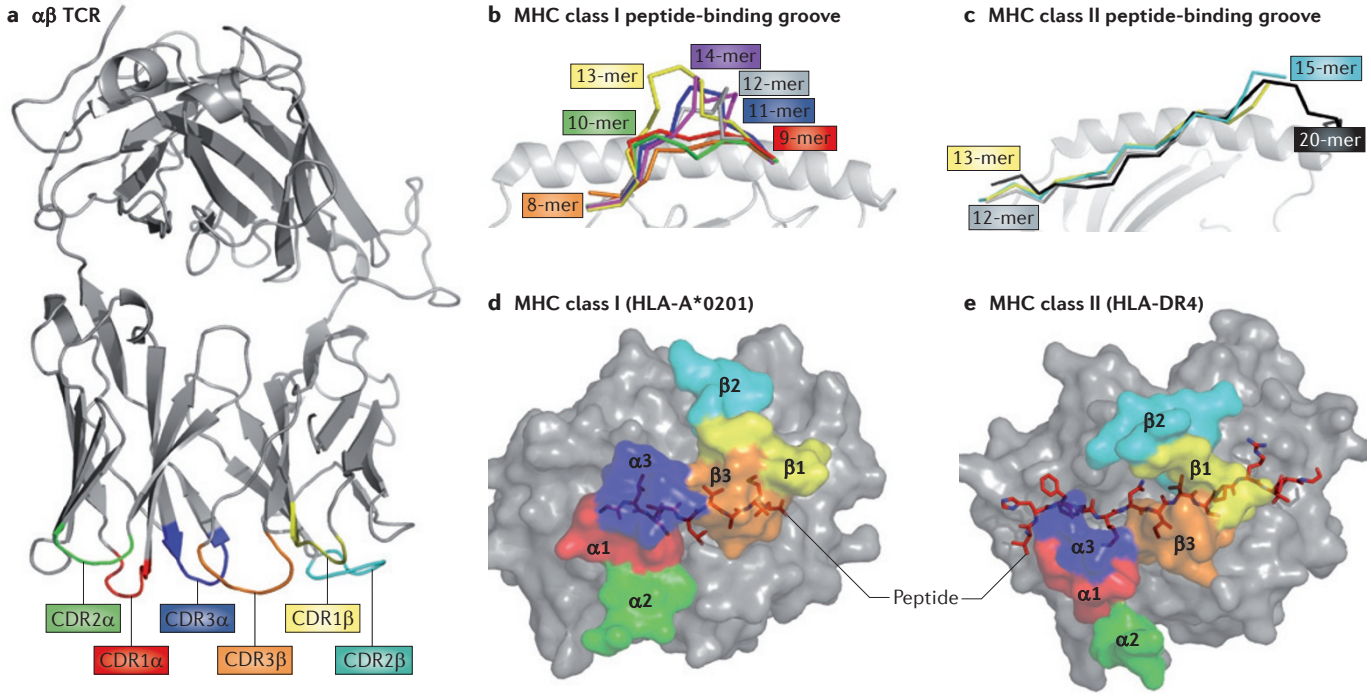

Figure 1 | **TCR and peptide–MHC structures.** **a** | Depicted is a ribbon model of an αβ T cell receptor (TCR) showing the positions of the six variable complementarity-determining region (CDR) loops. **b,c** | MHC class I and class II molecules can accommodate antigenic peptides of different lengths. The closed ends of the MHC class I binding groove cause long peptides to 'bulge' out of the binding groove, and this bulging increases with each additional amino acid in the peptide. By contrast, the ends of the MHC class II binding cleft are open, which allows the accommodation of much longer peptides without the need for peptide kinking. **d,e** | The images show HLA-A*0201 (in grey) presenting the immunodominant GLCTLVAML peptide (stick model) from Epstein–Barr virus and HLA-DR4 (in grey) presenting a peptide from myelin basic protein (MBP). TCRs dock on a peptide–MHC complex in a diagonal mode that is conserved for binding to MHC class I and class II molecules. The colours indicate the docking footprints of the AS01 TCR[96] and MSC-2C8 TCR[97] on their cognate peptide–MHC complexes and show the 'footprints' on the MHC complex of the six CDR loops. In general, the germline-encoded CDR1 and CDR2 loops interact mainly with the MHC molecule itself, whereas the hypervariable CDR3 loops sit over the peptide. However, the small structural database that has been compiled to date already contains examples in which CDR1 and CDR2 make substantial interactions with the peptide and in which CDR3 has an important role in contacting the MHC molecule[98,99].

The diversity of TCRs is based on the six complementarity-determining regions (CDRs), which engage both the peptide and the MHC molecule[5] (FIG. 1). Typically, MHC class I and class II molecules present peptides from endogenous and exogenous antigens, respectively. The MHC class I molecule has a closed-ended peptide-binding groove and binds peptides of 8–14 amino acids in length. Longer peptides become increasingly distorted in the central region of the MHC class I molecule as the peptide length increases, resulting in peptide 'bulging'[6,7]. By contrast, the ends of the MHC class II peptide-binding cleft are open, allowing even longer peptides to extend beyond this groove without bulging (FIG. 1b,c).

The clonal selection theory[8,9] proposed that individual lymphocytes are specific for a single antigen and that the recognition of alternative ligands is unlikely. For many years the concept of huge numbers of TCRs successfully providing immunity to all foreign peptides in a 'one-clonotype–one-specificity' paradigm was accepted. However, several workers questioned this concept[10–13]. Most notably, Don Mason called for the abandonment of such a notion in his seminal thesis on the topic (see REF. 10). Many of the reasons for this paradigm shift were based on the simple arithmetic of effective immunity requiring the recognition of $>10^{15}$ potential foreign peptides. Indeed, put in the context of 10^{15} T cells weighing >500 kilograms, the notion of immune coverage by a naive pool of 10^{15} monospecific TCRs as suggested by the clonal selection theory is clearly absurd[10]. There are only 10^{12} T cells in a human, and more recent studies have estimated that there are $<10^{8}$ distinct TCRs in the human naive T cell pool[14].

In humans, MHC molecules are encoded within the HLA locus. The HLA locus is the most polymorphic region of the human genome and is known to encode more than 7,000 allelic variants across the population, with a large number of these variants present at appreciable frequencies[15]. Some HLA loci are among the fastest evolving coding regions in the human genome[16]. Each individual expresses six different classical peptide-presenting HLA class I molecules (two HLA-A, two HLA-B and two HLA-C) and six HLA class II molecules (two HLA-DR, two HLA-DQ and two HLA-DP). The expression of a wide variety of HLA molecules ensures that individuals across the population present different antigenic peptides and provides the greatest chance that some individuals may survive any emerging infection. It is extremely difficult to link HLA diversity to past pandemics, but evidence of the importance of infectious diseases in driving HLA selection can be seen with current emerging infectious diseases. For example, homozygosity at HLA class I alleles results in faster disease progression during HIV infection[17], and some HLA class I alleles are associated with lower viral loads and protection from disease[18]. Various factors in addition to T cell immunity are thought to contribute to the maintenance of HLA diversity, including natural killer cell recognition[19], mate selection[20,21] and transmissible tumours[22]. Overall, the fact that mutations that alter the amino

acid sequence of HLA class I and class II molecules are clustered around the peptide-binding cleft and often alter the peptide sequence that is preferentially bound by the HLA molecule[23–25] strongly suggests that HLA diversity is upheld to increase the variety of peptides displayed.

The TCR recognizes peptide antigens presented by all HLA variants. Unlike the B cell receptor, the protein sequence of the TCR is fixed, and the TCR never undergoes affinity maturation. Thus, TCRs expressed by naive T cells are required to respond to all foreign antigens despite never having encountered them before and being unable to adapt to them at the protein sequence level. If the TCR repertoire was unable to recognize virtually all foreign peptides bound to self MHC molecules, then pathogens — which usually evolve many millions of times faster than their vertebrate hosts — would be expected to rapidly evolve to exploit these T cell 'blind spots' and overwhelm the host.

It is difficult to conceive of any obvious universal mechanism that might transmit knowledge of 'presentable' epitopes from previous infections between generations within the TCR CDR loops[10]. In the absence of 'prior knowledge' of the epitopes that might be encountered, T cell immunity must provide immune cover for all possible foreign peptides that contain appropriate anchors for binding to self MHC molecules[10]. This universal cover represents a major challenge to the immune system, as the possible array of peptides that can be manufactured from the 20 proteinogenic amino acids of a length that can bind to self MHC molecules is vast (>10^{15}) (BOX 1). In fact, the theoretical number of possible peptides that T cells might provide immunity to is even greater, as it is possible to raise specific T cell responses to peptides that contain amino acids with post-translational modifications, such as glycosylation[26], citrullination[27], phosphorylation[28,29], cysteinylation and dimerization[30,31]. Thus, the number of potential foreign peptide–MHC complexes that T cells might encounter dwarfs the number of TCRs available.

Here, I consider how the challenge of this disparity has been met by compromising on antigen specificity so that individual T cells are capable of responding to enormous numbers of different peptide–MHC complexes. This inevitable, extensive T cell cross-reactivity has some profound consequences, including providing a plausible cause for autoimmune disease. I also discuss how the consequences of TCR binding degeneracy offer substantial scope for multiple therapeutic interventions.

TCR binding degeneracy and structure

The recognition by TCRs of all HLA molecules and a roughly conserved diagonal mode of binding on peptide–MHC complexes suggest that TCR interactions conform to some 'rules of engagement' (FIG. 1). Such rules have been proffered in the form of a TCR 'interaction codon'[32] that interacts with MHC class II molecules, and in the form of a 'restriction triad'[7] that consists of three largely conserved residues in MHC class I molecules that interact with TCRs. These rules fit the generally observed arrangement of TCR–peptide–MHC interactions, in which the germline-encoded (that is, non-rearranged) CDR1α, CDR1β, CDR2α and CDR2β elements of the TCR contact the germline element of the MHC molecule, whereas the non-germline (that is, somatically rearranged) CDR3α and CDR3β loops contact the 'random' peptide element (FIG. 1). However, these convenient rules fail to match all the structures of TCR–peptide–MHC complexes that have been generated to date[5], and MHC mutational studies show that the dependency on fixed pairwise interactions between a TCR and a peptide–MHC complex varies widely between individual TCRs[33]. The peptide–MHC complex itself can also change its conformation following TCR binding[34–36]. Thus, it is clear that TCR–peptide–MHC interactions are not rigidly conserved but rather allow for considerable flexibility within the confines of some general orientation and binding rules.

The tumour-specific DMF4 TCR provides an excellent example of how large changes in TCR orientation can increase T cell cross-reactivity. The DMF4 TCR engages the nine-amino-acid (9-mer) peptide AAGIGILTV and the 10-mer peptide ELAGIGILTV (which have overlapping sequences) in the context of HLA-A*0201 by adopting a different orientation for the two peptide–MHC complexes[37]. TCR-binding plasticity can extend beyond different peptide binding registers or different peptide binding angles on peptide–MHC complexes because the CDR loops can be extremely flexible[38,39]. The mouse 2C TCR structure has been solved in complex with EQYKFYSV–H2-K^b (REF. 40), EQYKFYSV–H2-K^{bm3} (REF. 41), SIYRYYGL–H2-K^b (REF. 42) and

Box 1 | **Extensive T cell cross-reactivity and apparent specificity are not incongruous**

Peptide length	Total number of possible peptides	Number of MHC binders (if 1% of the total bind)	Number of MHC binders (if 3% of the total bind)
8-mer	2.6×10^{10}	2.6×10^{8}	7.8×10^{8}
9-mer	5.1×10^{11}	5.1×10^{9}	1.5×10^{10}
10-mer	1.2×10^{13}	1.2×10^{11}	3.6×10^{11}
11-mer	2.0×10^{14}	2.0×10^{12}	6.0×10^{12}
12-mer	4.1×10^{15}	4.1×10^{13}	1.2×10^{14}
13-mer	8.2×10^{16}	8.2×10^{14}	2.4×10^{15}
14-mer	1.6×10^{18}	1.6×10^{16}	4.8×10^{16}

From the 20 proteinogenic amino acids, it is possible to generate vast numbers of peptides of a length that can be presented by MHC molecules (see the table). T cells are specific because any given T cell can recognize only a tiny fraction of the 'universe' of peptides that can be presented by any given MHC molecule, but they are multispecific because the peptide universe is so large. By way of example, a T cell that recognizes 1 million 10-mer (10-amino-acid) peptides will have less than a 1 in 10 million chance of recognizing any 10-mer peptide chosen at random from the entire peptide universe. These numbers indicate that if a T cell that recognizes 1 million different 10-mer peptides was tested for recognition of random 10-mer peptides at a rate of 1 every minute then on average it would take over 20 years before a cross-reaction was seen! Even the total number of overlapping peptides that can be made from the entire human proteome is an extremely small fraction of all possible peptides (for example, fewer than 10^7 of the total possible number of 10-mer peptides (>10^{13}) can be made from the human proteome).

In the environment in which T cells function, the important number is the frequency of functional recognition of unrelated peptides that can be processed and presented by MHC molecules. Assuming that just 1% of possible peptides are presented by an MHC molecule, then the functional recognition of 10^6 10-mer peptides by a single TCR translates into a frequency of cross-reactivity of 1 in 100,000, which is in good accord with an experimental attempt to directly measure this parameter[95]. Thus, the sheer size of the possible peptide universe allows T cells to be enormously cross-reactive while appearing to be very specific within the environment in which they are required to operate.

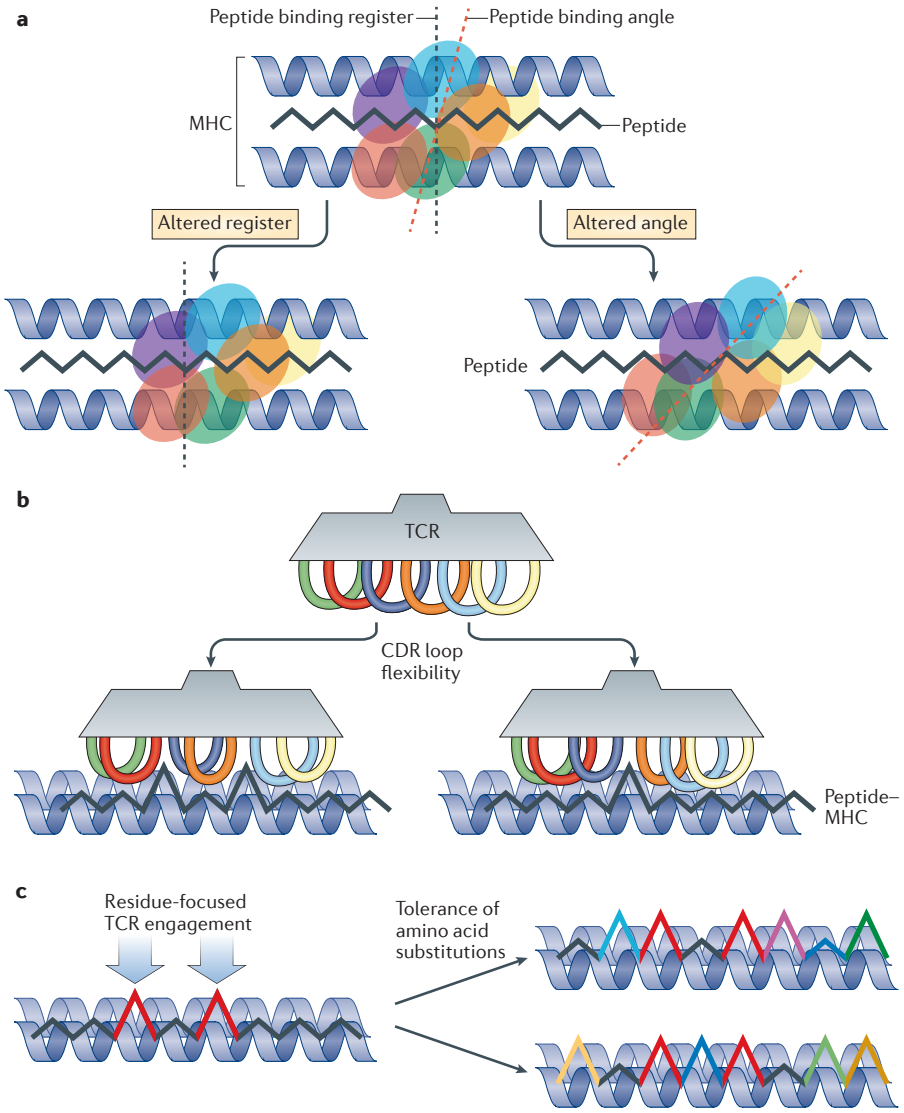

Figure 2 | **The TCR uses multiple mechanisms to engage numerous peptide–MHC molecules.** **a** | Macro-level changes enable the T cell receptor (TCR) to bind to peptide–MHC complexes with an altered peptide binding angle (red dotted line) and/or peptide binding register (black dotted line) within a roughly diagonal binding mode[38]. The cartoon shows 'footprints' of the TCR complementarity-determining region (CDR) loops projected down onto the peptide–MHC platform. **b** | Micro-level CDR loop flexibility enables the accommodation of different peptide–MHC 'landscapes'. The cartoon shows a side view of a TCR engaging a peptide–MHC complex. **c** | Structural studies show that most TCRs focus on two to four upward-facing peptide residues. In this example, the TCR is focused on the two peptide residues shown in red. Such residue-focused interaction allows the TCR to tolerate multiple amino acid substitutions at other positions in the peptide (indicated by different colours). The above examples are not mutually exclusive and represent only some of the possibilities. MHC-binding motifs often allow for different residues at primary MHC anchors[49]. It should also be noted that TCRs can change the conformation of the peptide–MHC complex following engagement[34–36].

QLSPFPFDL–H2-L^d (REF. 43). Although the 2C TCR adopts a similar general conformation on each of these ligands, it assumes a more diagonal binding orientation on the H2-L^d ligand, positioning its CDR1 and CDR2 loops over different regions of the MHCα1 and MHCα2 helices[43]. In a more extreme example of TCR plasticity, the YAe62 TCR can recognize disparate MHC class I and class II ligands by adopting alternative conformations[44]. The human A6 TCR provides another well-documented example of plasticity and can accommodate the removal of bulky residues or the insertion of positively charged residues at the middle of the TCR–MHC interface with the cognate Tax peptide from human T-lymphotropic virus 1 (REF. 39).

The recently described 1E6 TCR — which was isolated from a patient with type 1 diabetes and which recognizes residues 15–24 of the preproinsulin molecule (PPI$_{15–24}$) presented in the context of HLA-A*0201 (REF. 45) — does not undergo structural rearrangements following ligand binding[46] but is still hugely cross-reactive. Despite a rigid 'lock and key' binding mode, T cells expressing the 1E6 TCR respond to over 1.3 million 10-mer peptides at least as strongly as they respond to the PPI$_{15–24}$ peptide[46,47]. Peptides were identified that were >100-fold more potent than PPI$_{15–24}$ at activating 1E6 TCR-expressing T cells but that differed from PPI$_{15–24}$ at seven of the ten amino acid positions[47]. This promiscuity is explained by the structure of the 1E6 TCR–PPI$_{15–24}$–HLA-A2 complex, in which the TCR exhibits peptide-centric binding that is focused on just two amino acids in the peptide[46]. This residue-focused mode of binding presumably allows for substitutions at other positions that, in some cases, must considerably stabilize the interaction. In another example of such peptide-centric binding, a single amino acid interchange within two HIV envelope epitopes was shown to reciprocally swap the specificities of two CD8$^+$ T cell clones[48], suggesting that a dominant focus on a single amino acid residue in the peptide within a peptide–MHC complex might be reasonably common. Indeed, the TCR–peptide–MHC structures that have been described to date show that usually only a few upward-facing residues from the peptide contribute to the interaction of the TCR with the peptide–MHC complex. Thus, data from the limited number of TCR structures available indicate that TCRs can exhibit substantial binding degeneracy by being extremely flexible and/or through a focused interaction that is dominated by a few peptide residues (FIG. 2).

Together, this binding promiscuity at the TCR interface and the flexible MHC-binding 'motifs'[49] that often allow the accommodation of several amino acids at primary MHC anchor positions enable a substantial number of peptides to act as agonists for any given TCR.

T cells must be extremely cross-reactive

It is possible to generate vast numbers of peptides of the length recognized by T cells from the 20 proteinogenic amino acids (BOX 1). Even conservative estimates predict that substantially more than 1% of these peptides will possess anchors that allow them to bind to any single MHC molecule. Taking 10-mer peptides as an example, it is possible to generate >10^{13} different peptides of

10 amino acids in length from the 20 amino acids. Assuming that at least 1% (>10^{11}) of these peptides can bind to a given self MHC molecule, a heterozygous human antigen-presenting cell could theoretically present more than 12×10^{11} different 10-mer peptides on its six MHC class I molecules and six MHC class II molecules. Furthermore, as MHC class II molecules can present longer peptides that can 'frame-shift' within the open-ended binding groove (FIG. 1), Mason calculated that each MHC class II molecule could theoretically present almost 10^{17} different 14-mer peptides, assuming that 3% of all peptides associate with MHC class II molecules[10], and this is without even considering the possibility of post-translational modifications. In summary, the number of potential peptide antigens exceeds the number of TCRs available to respond to them by many orders of magnitude, so T cells can only provide comprehensive immune cover if each one is capable of recognizing many peptides.

T cells are extremely cross-reactive. The theoretical arguments of Mason suggesting that T cells must each recognize on average at least 1 million individual peptides[10] have recently gained traction as a result of data that demonstrate this level of cross-reactivity and provide plausible structural mechanisms for its occurrence. All T cells are 'auditioned' in the thymus and only those that react weakly with a self peptide–MHC ligand are positively selected[50]. T cells bearing TCRs that react strongly to self antigens are 'culled' at this stage.

Extensive TCR binding degeneracy and cross-recognition of peptide–MHC molecules by thymocytes has been elegantly demonstrated by studies showing that a remarkably comprehensive T cell repertoire can be selected by a single peptide[51] and that the resulting T cells can be activated by peptides that are unrelated in sequence to the peptide that they were selected on[52]. Further compelling evidence that T cells can exhibit extensive cross-reactivity comes from studies with combinatorial peptide libraries that comprise almost all possible peptides of a particular length[11,47,53–56]. These libraries are usually used as a series of sub-libraries laid out in positional-scanning format such that there is a sub-library with each amino acid fixed in each position and with all other positions made up of an equimolar mix of the remaining amino acids (of note, cysteine is generally excluded from the 'random' positions to avoid problems of oxidation) (see Supplementary information S1 (figure)). Studies with these libraries in T cell activation assays indicate that agonist ligands can contain several different amino acids at many positions. Several studies have gone on to use this approach to prove the 'Mason hypothesis' and show that individual T cell clones really can recognize over a million different individual peptides in the context of a single MHC molecule[47,56,57].

Control of T cell cross-reactivity. The antigen sensitivity of a T cell and its ability to respond to weaker TCR ligands are inexorably linked. T cell sensitivity to an antigen is not a fixed parameter. Memory T cells can recognize concentrations of a peptide antigen that are >50-fold lower than those recognized by naive T cells[58,59], and individual T cell clones can generate progeny with both high and low antigen sensitivities[60]. Antigen sensitivity can be regulated by changes in TCR expression levels or clustering on the cell surface, by changes in the expression or function of co-stimulatory molecules, by differential control of phosphatase pathways that dampen T cell signalling or by alterations in the glycosylation status of the TCR or other cell-surface molecules (reviewed in REF. 61). Although these mechanisms may regulate the antigen sensitivity of T cells, and thus the ability of T cells to cross-recognize weak TCR ligands, it is difficult to conceive how they might be used to tune the biophysics of TCR engagement with a specific ligand. By contrast, the CD4 and CD8 glycoproteins have a unique role in 'co-receiving' peptide–MHC molecules by binding to largely invariant sites on MHC class II and MHC class I molecules, respectively[62]. Thus, these co-receptors might possess an ability to differentially regulate the responsiveness of the TCR to the ligand and thereby modulate TCR specificity[63]. Indeed, CD8 is known to affect both the on-rate[64,65] and off-rate[66,67] of TCR–peptide–MHC class I engagement and therefore can modulate the kinetics of TCR binding by different peptide–MHC ligands. We have demonstrated how the strength of the peptide–MHC class I–CD8 interaction can have substantial effects on T cell cross-reactivity[53]. It is important to realize that, although the TCR sequence is invariant, TCR sensitivity to agonist ligands (and therefore T cell cross-reactivity) is not fixed and can be varied throughout development by a number of parameters[53].

Consequences of T cell cross-reactivity

The idea that immune cover is provided by limited numbers of highly cross-reactive T cells has both positive and negative implications. The presence of pools of cross-reactive T cells that each recognize large numbers

Glossary

Altered peptide ligands
(APLs). Peptide analogues that are derived from an original antigenic peptide. They commonly have amino acid substitutions at residues that contact the T cell receptor (TCR) and alter TCR engagement, resulting in different activation consequences than those induced by the wild-type ('index') antigenic peptide.

Antigen sensitivity
A measure of how sensitive T cells are to the density of cognate antigen on the antigen-presenting cell surface. T cell receptor (TCR) affinity for a peptide–MHC complex has a large role in antigen sensitivity, but the parameter is also affected by the expression of other molecules that influence cell–cell contact or the downstream signal transduction that results from TCR–peptide–MHC engagement.

Clonal selection theory
A theory proffered by Niels Jerne which states that there is already a vast array of lymphocytes in the body before any infection. Any challenge with antigen selects, and clonally expands, a single corresponding lymphocyte (B cell or T cell) from the pre-existing lymphocyte pool of differing specificities, and this clonal lymphocyte population then eliminates the antigen.

Complementarity-determining regions
(CDRs). The regions within antigen receptors that complement the shape of an antigen. The CDRs are the most variable part of the antigen receptor and are largely responsible for the diversity in these molecules. The CDRs allow antibodies and T cell receptors to recognize a vast repertoire of antigens.

Heterologous immunity
The term used to describe how an immune response to a pathogen can provide immunity to a non-identical pathogen. Heterologous immunity can be mediated by cross-reactive T cells or antibodies.

Molecular mimicry
Resemblance between epitopes contained in microbial and host proteins, leading to cross-reactivity of T cells in the host.

Original antigenic sin
A 'footprint' of immune responses is established during the first exposure to a pathogen. These specific memory T cell populations are preferentially re-expanded when re-exposed to the same antigen or one that is similar, thereby limiting the clonal expansion of new antigen-specific T cells. A similar mechanism has been proposed for B cell responses.

T cell cross-reactivity
The reaction of T cells to more than one distinct peptide–MHC ligand.

TCR binding degeneracy
Refers to the promiscuity of T cell receptor (TCR) engagement that allows a single TCR to bind to different peptide–MHC complexes.

of peptides but that do not respond to self peptides in the periphery has a number of positive consequences. First, a cross-reactive T cell repertoire generates a near perfect solution to the huge challenge of providing effective immune cover by allowing a limited number of T cells to provide immunity against virtually all foreign peptides that can bind to self MHC molecules. Second, a system with a limited number of hugely cross-reactive T cells is both temporally and spatially favourable, as far fewer T cells are needed to scan any infected cell than if the clonal selection theory was rigidly upheld. Third, the corollary of extensive T cell cross-reactivity is that several TCRs are likely to recognize any one peptide (and thus that T cell responses are polyclonal). Polyclonal recognition of peptide–MHC molecules makes it substantially more difficult for pathogens to escape immune recognition, as a mutation that escapes recognition by one TCR might be recognized by another. Fourth, extensive T cell cross-reactivity also provides excellent conservation of resources by generating 'one weapon with several triggers'.

Several documented examples show that an individual T cell clone can target more than one infection through different peptides, a phenomenon known as heterologous immunity[68]. Heterologous immunity between related pathogens is common. It is well known that immunity to cowpox provides cover for smallpox[69], and the tuberculosis vaccine bacterium *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) can provide some protection against leprosy[70]. But, the existence of extensive T cell cross-reactivity means that heterologous immunity can extend beyond the cross-recognition of pathogens with high sequence similarity to allow, for example, BCG-induced T cells to also provide immunity against poxviruses[71]. Similarly, $CD8^+$ T cells specific for the human papillomavirus HLA-A2-restricted YMLDLQPET peptide also recognize the HLA-A2-restricted TMLDIQPED peptide from coronavirus[72]. Indeed, $CD8^+$ T cell-mediated heterologous immunity can extend to very dissimilar antigens. For example, cells that are specific for the immunodominant GILGFVFTL peptide from influenza virus can often recognize the Epstein–Barr virus epitope GLCTLVAML[73] or the immunodominant HIV-derived SLYNTVATL antigen[74] (all of which are HLA-A2 restricted).

The extent of heterologous immunity and its importance to human immunity is not yet fully known. The potential positive outcomes of this phenomenon are clear, but heterologous immunity could also have deleterious effects. Documented negative consequences of heterologous immunity include influenza-specific $CD8^+$ T cells contributing to lymphoproliferation in Epstein–Barr virus-associated mononucleosis[75] or cross-recognizing a peptide derived from hepatitis C virus (HCV)[76], which increases the severity of HCV-associated liver pathology[77]. It is also possible that heterologous immunity via T cell cross-reactivity could encourage a suboptimal response to the second pathogen owing to 'original antigenic sin'. This antigenic sin could extend beyond the simple case of suboptimal sensitivity to the second antigen to a situation in which the original antigen has established a T helper 1 (T_H1)- T_H2- or T_H17-type response bias that is inappropriate for the second pathogen.

However, the most obvious and detrimental consequence of T cell cross-reactivity to vast numbers of individual peptides is the potential such a system has for causing autoimmunity (FIG. 3). Although strongly self-reactive T cells are deleted in the thymus[50], weakly cross-reactive T cells may survive and become activated in the periphery through the cross-recognition of peptides from infectious agents, a phenomenon known as molecular mimicry[78–81]. Memory T cells can be stimulated by peptide concentrations more than 50-fold lower than those required to stimulate naive T cells[58,59]. It is therefore likely that a memory T cell could be stimulated by a cross-reactive peptide with an affinity for the TCR that is far lower than that of the original pathogen-derived peptide. In such a situation, pathogen-mediated priming would be obligatory before functional cross-recognition of a self peptide, a notion that is consistent with the observation that infection can precipitate autoimmune diseases[79,82].

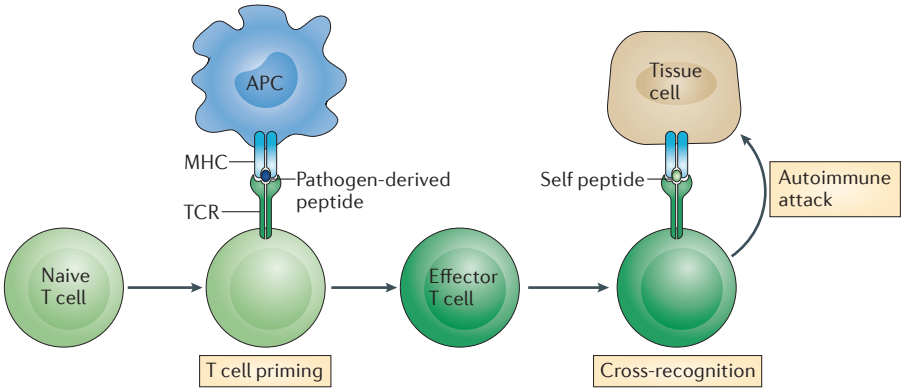

Figure 3 | **T cell cross-reactivity causes autoimmunity.** T cells expressing autoreactive T cell receptors (TCRs) are able to bypass system 'safety checks' and populate the periphery. Such T cells generally remain harmless. However, if such T cells become activated in response to a pathogen-derived peptide and become effector T cells, they may then cross-recognize a self-derived peptide to cause autoimmune disease. APC, antigen-presenting cell.

Future therapeutic perspectives

The compromise imposed by T cells being hugely cross-reactive in order to provide complete immune cover dictates that an individual TCR–peptide–MHC pairing is highly likely to be suboptimal. Thus, it should be possible to improve the binding of any given TCR to its cognate antigen by enhancing the specific molecular matching. Indeed, yeast display[83], phage display[84] and computational design[85,86] have been used to produce TCRs that bind to peptide–MHC complexes with extremely high affinities (K_d <10 pM) and half-lives of many hours. The MHC class I pathway is predicted to present at least one peptide at the cell surface from every internally produced protein[10]. This allows TCRs to potentially target any cell based on its expression of any protein (FIG. 4a). Consequently, TCRs might have considerable advantages over regular antibody-based therapies, as they can target a substantially greater number of cellular proteins. Furthermore, there is now substantial evidence that it is possible to improve the affinity of almost any peptide antigen for a given natural TCR. Thus, there is ample scope for the rational design of therapeutic interventions that exploit the fact that most natural TCR–peptide–MHC interactions can be improved upon.

Enhanced TCRs in TCR gene transfer therapy. The rigours of thymic selection ensure that natural TCRs bind to ubiquitous self or tumour-associated antigens with substantially lower affinities than they bind to pathogen-derived antigens[87]. Natural TCR–peptide–MHC interactions have affinities (measured in terms of K_d) in the

range of 0.1–500 μM[87,88]. Within this range of TCR binding affinities, the affinity and/or half-life correlates with antigen sensitivity[65,89], placing natural antitumour T cells at a distinct disadvantage compared with their pathogen-reactive counterparts.

The transfer of TCR genes into recipient host T cells followed by the adoptive transfer of the T cells to patients allows the passive transfer of immunity and can provide a useful mechanism for breaking tolerance to tumour antigens[90]. This strategy has already shown some promise in patients with malignant melanoma[91], but there is room for improvement. The transfer of genes encoding TCRs that have been affinity matured to bind to tumour-associated peptide–MHC complexes with affinities as high as those of the best antiviral T cells (K_d = 100 nM)[87,88] could provide 'virus-like' tumour immunity. This process can also be used to generate TCRs with immune 'foresight', as demonstrated by the development of TCRs that could recognize all known escape variants of HIV-1 (REF. 88).

Enhanced TCRs as soluble therapies. High-affinity soluble TCRs provide an efficient means for the cellular targeting of intracellular antigens that are presented by MHC molecules *in vivo* (FIG. 4a). Soluble TCRs can be linked to other molecules, such as antibody Fab fragments, and can deliver these molecules to sites of antigen expression *in vivo*[92]. Despite the low copy number of most peptide–MHC molecules (<50 copies per cell), we have recently used a soluble TCR fused to a CD3-specific Fab fragment to induce tumour regression *in vivo*[92]. These bispecific T cell-engaging TCRs function by recruiting polyclonal T cells via the CD3-specific Fab component but do not by themselves crosslink TCRs or induce T cell activation. Once these molecules are bound to a target cell surface, they become potent activators of antigen-experienced CD8$^+$ T cells and promote the lysis of targets expressing as few as ten cognate peptide–MHC complexes[92] (FIG. 4b). A similar approach could be used to dampen autoimmunity by crosslinking inhibitory receptors such as cytotoxic T lymphocyte antigen 4 (CTLA4).

Enhanced T cell ligands (TOPSORT). The fact that any TCR will be capable of recognizing enormous numbers of ligands paves the way for therapies based on altered peptide ligands (APLs). APLs can have advantages over natural ligands, as they can bind strongly to TCRs and can break tolerance to self ligands (including tumour-derived ligands). Previous assumptions about APLs, such as the suggestion that altering a buried anchor residue will not substantially alter TCR binding, have proved to be incorrect[93]. Nevertheless, combinatorial screening of peptide (or non-peptide) ligands can be used to determine the preferred binding 'landscape' of any TCR and circumvent the requirement for any assumptions. The nature of the system makes it highly likely that each TCR has a different preferred binding landscape. This then enables relatively precise targeting of specific TCRs within populations of antigen-specific T cells through a process termed TCR-optimized peptide skewing of the repertoire of T cells (TOPSORT), which can be used to sort the most effective clonotypes (FIG. 5). The widespread applicability of

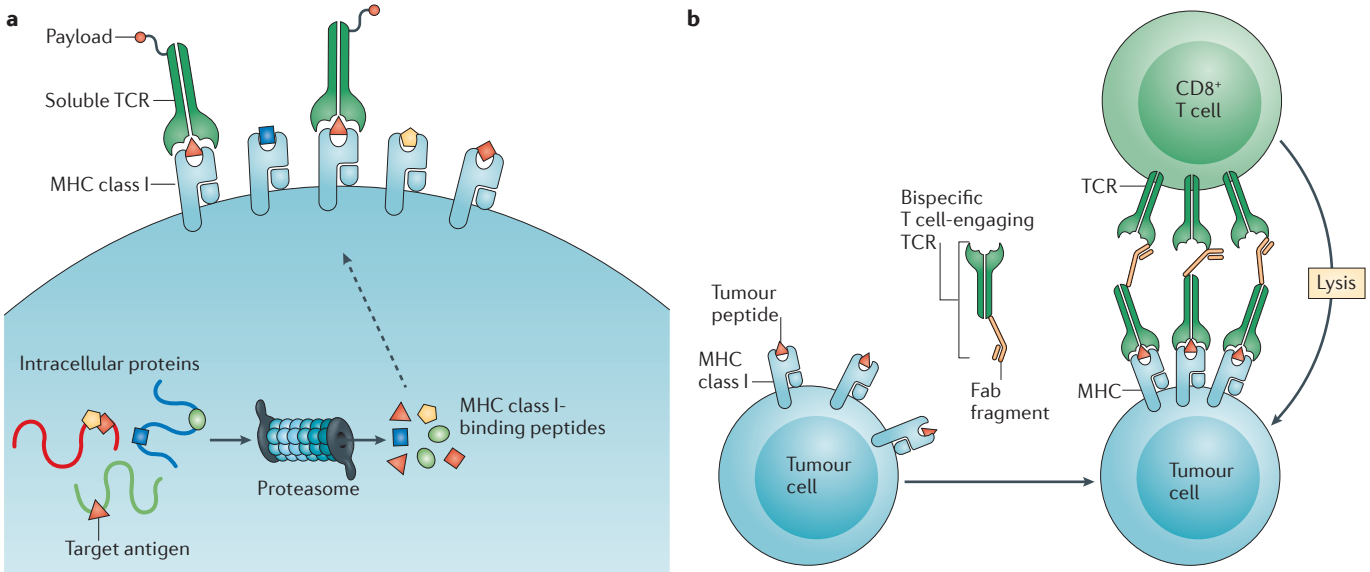

Figure 4 | **Enhanced TCRs as soluble therapies. a** | The MHC class I presentation pathway presents peptides at the cell surface from intracellular proteins. This potentially allows soluble high-affinity 'monoclonal' T cell receptors (TCRs) to target any cell based on its expression of any protein. 'Monoclonal' TCRs are able to use the MHC class I presentation pathway to 'see inside' cells and scan them for internal anomalies. This 'X-ray vision' opens up access to a far greater range of disease-relevant antigens than are available for monoclonal antibodies. TCRs can be engineered to deliver a variety of molecules that stimulate or suppress the immune system. Potential 'payloads' include antibody Fab fragments that then deliver a signal to immune cells. As MHC-bound peptides are often present at low copy numbers (<50 copies per cell), the payloads delivered by TCRs must act at very low concentrations. **b** | High-affinity tumour-specific TCRs that are manufactured as bispecific T cell-engaging molecules by linking them to CD3-specific Fab fragments can direct the lysis of tumour cells by CD8$^+$ T cells and thereby induce the regression of established tumours[92]. These molecules do not activate T cells as monomers at the concentrations used. T cell-engaging TCRs bind to the cognate antigen on the tumour cell surface with long half-lives and 'present' the linked CD3-specific Fab fragments. These Fab fragments then crosslink TCRs on the surface of antigen-experienced CD8$^+$ T cells, resulting in cellular activation and elimination of the target cell[92]. The delivery of toxins with soluble TCRs is not recommended, as the soluble TCR constructs are taken up by scavenging cells such as macrophages. Thus, molecules that deliver a particular signal to a specific effector cell are preferable. For example high-affinity TCRs could be used to downregulate immune responses by signalling through inhibitory receptors such as cytotoxic T lymphocyte antigen 4 (CTLA4) (not shown).

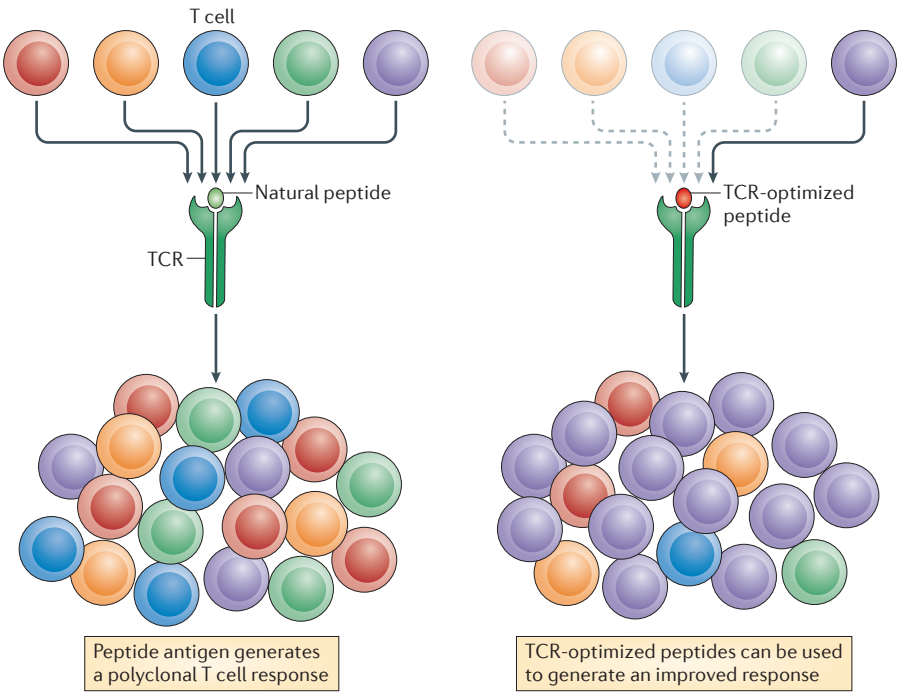

Figure 5 | **TCR-optimized peptide skewing of the repertoire of T cells.** Clonotypic T cell receptors (TCRs) that recognize the same antigen are not all equal, and one TCR may provide the most effective immunity. In the case of HIV for example, one TCR may be more difficult for the virus to escape from than other TCRs. If the required TCR is public (that is, it occurs in all individuals with the restricting HLA molecule) or has a public-type motif, then a TCR-optimized peptide for this clonotype could be used to skew the response towards the most effective clonotype(s). There are no known rules that enable the prediction of which TCRs a particular ligand will stimulate. Thus, this process requires pre-testing using *in vitro* priming assays to ensure that it induces the required clonotype(s) while minimizing the induction of suboptimal clonotypes.

this approach is dependent on the effective clonotype being 'public'[94] (that is, occurring in all individuals with the restricting HLA molecule) or having a public motif that is shared by all individuals with the relevant HLA molecule. Our own preliminary studies using *ex vivo* peripheral blood mononuclear cells show that this approach can be used to skew the clonotypes that respond to a tumour antigen (J. Ekeruche-Makinde *et al.*, unpublished observations). A similar approach could be used to skew the clonotypes induced by a vaccination against HIV towards those that are known to be more difficult for HIV to escape from.

Concluding remarks

Accumulating evidence, including direct estimates of the total number of TCRs in a human, supports Mason's notion that we should abandon the 'one-clonotype–one-specificity' paradigm suggested by clonal selection theory in favour of a 'one-clonotype–millions-of-specificities' reality. The simple arithmetic of T cell immunity allows T cells to be highly cross-reactive while appearing to be exquisitely specific in the environment in which they are expected to function (BOX 1). However, the realities of T cell immunity dictate that TCRs are very rarely an optimal fit for a real antigen and that real MHC-presented peptide antigens are rarely the optimal agonists for a given TCR. This compromise provides multiple opportunities for rational therapeutic interventions based on the directed manipulation of T cell immunity.

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Competing interests statement

The author declares no competing financial interests.

FURTHER INFORMATION

Andrew K. Sewell's homepage: http://www.tcells.org

SUPPLEMENTARY INFORMATION

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