TIMELINE

Interrogating the repertoire: broadening the scope of peptide–MHC multimer analysis

Mark M. Davis, John D. Altman and Evan W. Newell

Abstract | Labelling antigen-specific T cells with peptide–MHC multimers has provided an invaluable way to monitor T cell-mediated immune responses. A number of recent developments in this technology have made these multimers much easier to make and use in large numbers. Furthermore, enrichment techniques have provided a greatly increased sensitivity that allows the analysis of the naive T cell repertoire directly. Thus, we can expect a flood of new information to emerge in the coming years.

Until the 1960s, all lymphocytes were thought to be the same. However, in that decade it became apparent that there are distinct lymphocyte types — that is, B (bursa-derived) cells and T (thymus-derived) cells — with very different characteristics. Subsequently, the development of monoclonal antibodies and the fluorescence-activated cell sorter made possible the identification of many subtypes of lymphocytes and the analysis of their developmental characteristics, and new subsets are still found on a regular basis. One very important way to subdivide lymphocytes is by their antigen specificity. In B cells, this is relatively straightforward, and a number of studies have shown that haptens or whole proteins with appropriate labels can be used to track the development of a specific antibody response[1–4]. However, the affinity of the T cell receptor (TCR) for peptide-loaded MHC is usually so low (dissociation constant ~50 μM; 10,000-fold weaker than a typical antibody–antigen interaction) that it was clear from early measurements[5] that a labelled monomeric peptide–MHC reagent would not survive even a single washing step (FIG. 1a). This led some of us (J.D.A. and M.M.D.) and colleagues to try different ways of multimerizing peptide–MHC complexes to improve their binding characteristics. Ultimately, this led us to adopt a site-specific biotinylation method[6], by which peptide–MHC complexes could be tetramerized with fluorescently labelled streptavidin molecules.

The first MHC tetramers were made with the mouse MHC class II molecule I-E^k complexed with a cytochrome *c*-derived peptide, and we later used HLA-A*0201 for the identification of HIV-specific $CD8^+$ T cells (in collaboration with A. McMichael, J. Bell and others)[7]. Such MHC tetramers have proved to be a remarkably useful way to label most αβ T cells and γδ T cells, in particular for flow cytometric analysis and even for *in situ* staining. Moreover, other multimeric forms have been developed, including dimers[8], pentamers, lipid vesicles and dextramers. All of these forms contain multiple peptide–MHC complexes or other T cell ligands that form multiple bonds with TCRs to achieve stable binding and, therefore, can be used to label and purify T cells of a particular specificity. This principle is illustrated in FIG. 1, which compares monomer binding with tetramer binding.

The value of directly labelling antigen-specific T cells has been substantial. For example, it has proved to be a much more accurate method of quantifying the development of an antigen-dependent response than limiting dilution cloning[9]. Tetramers have also been used to quantify the relative off-rates for TCR binding in bulk or at the single cell level[10] by monitoring the decay of tetramer staining, while blocking rebinding with MHC-specific antibodies. For $CD8^+$ T cells, the off-rates for TCR binding can be measured even more accurately by using tetramers of an MHC class I molecule deficient in CD8 binding[11]. In addition, tetramers enable the physical purification of antigen-specific T cells by flow cytometry and, perhaps most importantly, the identification of T cells with a given specificity regardless of their biological activity. For example, anergic T cells can be detected, despite their lack of proliferation or cytokine production[12]. These characteristics of peptide–MHC tetramers — aided by the development of the US National Institutes of Health (NIH) Tetramer Core Facility and the commercial availability of tetramers through Beckman Coulter, ProImmune and Immudex — have led to their wide usage in T cell research. Peptide–MHC tetramer-based applications include basic and clinical research into vaccine development, infectious diseases, autoimmunity and cancer responses.

Recently, a number of advances in peptide–MHC tetramer staining technology have opened up new possibilities in research. These developments include: the generation of MHC tetramers with exchangeable peptides, which greatly simplifies the production of hundreds or thousands of tetramers from one batch of prepared MHC protein[13]; an enrichment procedure that has allowed the characterization of very rare T cells[14,15], even those in the naive repertoire; tetramer-guided epitope mapping[16]; and combinatorial staining techniques that allow many more tetramers to be used simultaneously[17,18]. In addition, a long-standing difficulty in staining with some MHC class II-based tetramers has been overcome, at least partially, by fixing peptides in a defined register within the MHC molecule binding groove using a disulphide bond to the MHC molecule, to prevent them from sliding into different registers[19,20] (TIMELINE).

In this Review, we summarize the basics of current peptide–MHC multimer usage, with a particular emphasis on these new techniques and their application to T cell biology.

Improvements in peptide–MHC multimers

Until recently, making more than a handful of tetramers at a time was very difficult, as each tetramer required a separate manufacturing and purification scheme. The development of exchangeable peptides has changed the situation radically, especially for MHC class I tetramers and at least partially for MHC class II. For the generation of peptide–MHC class I tetramers, Schumacher and colleagues introduced the use of ultraviolet (UV)-sensitive peptides (FIG. 2), which are MHC class I-binding peptides that contain a UV-sensitive residue. A brief pulse of UV radiation induces this residue to break the peptide into two pieces, and this causes the peptide fragments to dissociate from the MHC molecule and allows an intact peptide in solution to bind in their place. Thus, for a given MHC allele, a single UV-sensitive peptide–MHC complex can be made and purified using standard methods[13] and then aliquoted into hundreds or even thousands of different peptide solutions to create many different tetramers[21].

The production of MHC class II tetramers with exchangeable peptides is more difficult, at least in part because the peptide-binding site is made from two different polypeptides, instead of one as in the MHC class I molecule. The best hope for exchangeable peptide–MHC class II tetramers comes from Wücherpfennig and Ward and their colleagues, who have expressed HLA-DR or I-A^u molecules as fusion proteins with the class II-associated invariant chain peptide (CLIP), separated by a linker region that incorporates a protease cleavage site[14]. Following cleavage, CLIP rapidly dissociates and can be replaced by a synthetic peptide of interest. This method works well for many HLA-DR alleles but may not work as well for HLA-DQ or HLA-DP alleles.

Labelling T cells specific for non-classical MHC molecules. CD1d is an MHC class I-like molecule that is the restricting element for responses by natural killer T (NKT) cells. NKT cells recognize glycolipid antigens of either microbial or self origin[22] that are bound to CD1d. Recombinant CD1d molecules have been produced in *Escherichia coli*[23], in insect cells transduced with baculoviral vectors[24] and in human embryonic kidney 293T cells transduced with lentiviral constructs[25]. The NIH Tetramer Core Facility also uses a lentiviral vector for expression of CD1d (unpublished; not identical to the system used by Li *et al.*[25]), as well as to produce CD1a, CD1b and CD1c molecules for use in tetramers. Moreover, tetramers have been constructed with the non-classical MHC molecules H2-T10, H2-T22, MHC class I polypeptide-related sequence A (MICA) and MICB, which are differentially expressed as part of the cellular stress response. Chien and colleagues have made tetramers of the mouse H2-T10 and H2-T22 molecules in order to label specific γδ T cells[26]. Similarly, the Spies group has generated tetramers of MICA to stain γδ T cells in humans[27].

Non-MHC tetramers. The potential of tetramer technology has inspired many investigators to apply it to molecules other than peptide–MHC complexes. Similarly to the peptide–MHC tetramers, tetramers of various proteins provide the advantage of cooperative binding and can be used to detect expression of their cognate ligands. TCR tetramers have been produced and used for MHC typing in rhesus macaques[28] and to screen peptide–MHC molecule libraries[29]. Killer cell lectin-like receptor subfamily G, member 1 (KLRG1) tetramers were used to identify cadherins as ligands for KLRG1 (REF. 30). Moreover, natural killer group 2, member D (NKG2D) tetramers were used to identify NKG2D ligands[31], and tetramers have also been generated with multiple ligands of NKG2D[32]. Altman and colleagues produced tetramers of CC-chemokine ligand 19 (CCL19) and used them to identify lymphocytes expressing CC-chemokine receptor 7 (CCR7)[33]. Thus, the range of applications is almost endless. TABLE 1 summarizes the production methods and applications of the various types of protein tetramers.

Other peptide–MHC multimer formats. Several other peptide–MHC multimer formats have been developed, all based on the principle of increasing the valency of the peptide–MHC complex to two or more to improve binding stability. The first peptide–MHC multimers to be produced after the initial work on tetramers were the MHC class I reagents of Schneck and colleagues[8] (commercialized as DimerX by BD Biosciences). These give less-robust staining than tetramers in many cases. Peptide–MHC multimers with a valency of five (pentamers) are available from ProImmune, and it is claimed that this format provides increased sensitivity to low-affinity TCRs owing to the greater valency. Other approaches include fluorescently labelled vesicles coated with peptide–MHC complexes[34]. Streptavidin-coated quantum dots have also been used and should have increased valency compared with standard tetramers, in addition to having improved fluorescent properties[35] (described below). More recently, peptide–MHC complexes linked to fluorescently labelled polydextran (dextramers; commercially available from Immudex) have proved to be useful for the detection of T cells with low-affinity TCRs because of their high valency[36].

As for interesting alternatives to peptide–MHC tetramer staining, our group[37], as well as Heath and colleagues[38], have developed peptide–MHC molecule microarrays for high-throughput analysis of antigen-specific T cells. But, a major limitation of this approach so far has been that the capture efficiency is very low (<5% of targeted cells), so it can only detect the most abundant specificities.

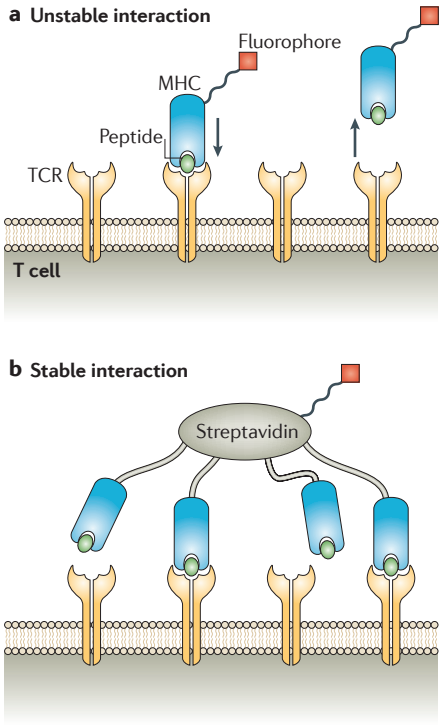
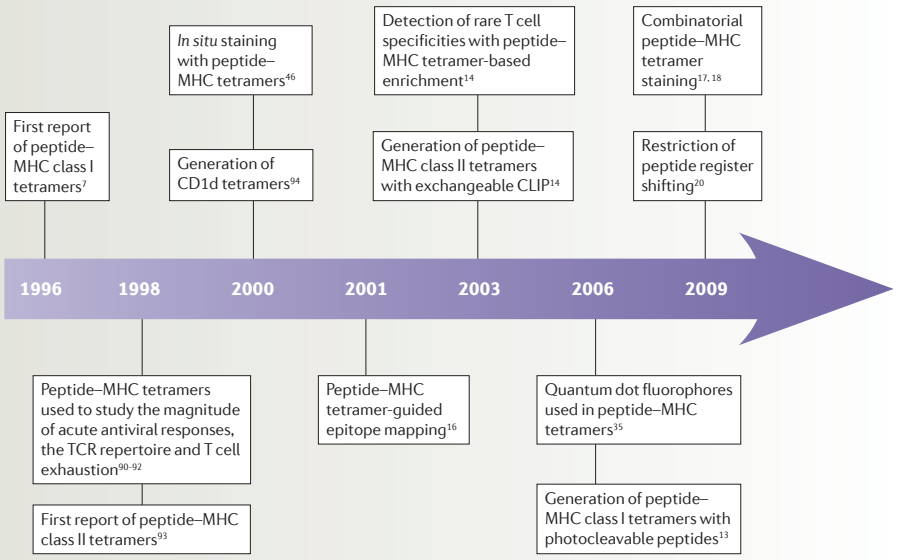


Figure 1 | **The advantage of peptide–MHC tetramers and other multimers for the detection of antigen-specific T cells. a** | As T cell receptors (TCRs) typically dissociate quickly from peptide–MHC complexes (with half-lives of a few seconds), fluorescently labelled monomeric peptide–MHC molecules do not normally survive the washing step during a staining procedure. **b** | By contrast, if two or more ligands that are part of a tetramer bind simultaneously, then even when one dissociates, others keep the tetramer bound to the cell.

Timeline | **Overview of peptide–MHC-related technological developments**



CLIP, class II-associated invariant chain peptide; TCR, T cell receptor.

Peptide–MHC multimers in flow cytometry

When using peptide–MHC tetramers in flow cytometry, it is crucial to choose an appropriate method to validate the specificity of tetramer-stained cells, and this depends on the experimental setup. Usually it is good to start with the use of negative control tetramers conjugated to a different fluorophore. Then, more definitive controls are also important, such as the assessment of TCR variable region usage skewing and of peptide-induced TCR downregulation. In addition, following cell sorting, TCR sequences can be analysed and TCR genes can be transferred from the candidate cells into immortalized T cell lines to show that the specificity can be reconstituted.

For the flow cytometric analysis, after all of the appropriate controls are included, only a limited number of fluorescence channels remain for the assessment of TCR specificities or phenotypic markers. In addition, a confounding factor that is particular to the use of peptide–MHC tetramers is the empirically determined fact that only a handful of commercially available fluorophore–streptavidin conjugates can provide tetramers that are effective for cell staining. The most common fluorophores used to label peptide–MHC tetramers are phycoerythrin and allophycocyanin, which have been chosen for their brightness on commercial flow cytometers and for their robust ability to form tetramer reagents that consistently provide high-quality staining. To our knowledge, none of the small-molecule fluorophore–streptavidin conjugates can be used for tetramer production. It was recently suggested that this is due to the loss of valency that occurs as a consequence of fluorophore conjugation to a crucial lysine residue in the biotin binding pocket of streptavidin[39]. By contrast, larger fluorophores, such as phycoerythrin, allophycocyanin and their analogues, are typically conjugated to streptavidin with a one-to-one labelling stoichiometry, which has little impact on streptavidin valency. The disadvantage of small-molecule fluorophores can be overcome by using a modified form of streptavidin that has a cysteine residue at the carboxyl terminus of each subunit, as the fluorophore can then be coupled to this residue using thiol-reactive chemistry[39].

The use of quantum dots — which display low background staining following polyethylene glycol coating and can be applied for combinatorial peptide–MHC tetramer staining — may also greatly ease the spectral constraints that exist for tetramer staining experiments. Quantum dots are fluorescent semiconductor nanocrystals that have a broad excitation spectrum and a narrow emission spectrum, the peak wavelength of which is a function of the size of the nanocrystal. Streptavidin-coated fluorescent nanocrystals are available from several commercial suppliers, with emission wavelengths ranging from 525 to 800 nm. Because of their relatively narrow emission spectra, it is possible to use 4–5 spectrally distinct quantum dots in a single experiment, with a corresponding number of peptide–MHC tetramer specificities.

Combinatorial tetramer staining. Until recently, investigators have used tetramers of one colour to detect each TCR specificity. However, Newell *et al.* and Hadrup *et al.* have recently described the use of combinatorial methods, in which each T cell population of the same TCR specificity is stained with a mix of identical tetramers that are conjugated to different fluorophores[17,18]. A distinct combination of fluorophores is used for each T cell specificity, such that each population can be individually identified. In the approach taken by Hadrup *et al.*[18], only two fluorophores were used, which helped to reduce nonspecific staining but also reduced the total number of T cell specificities that could be probed. Either approach enables the detection of a greater

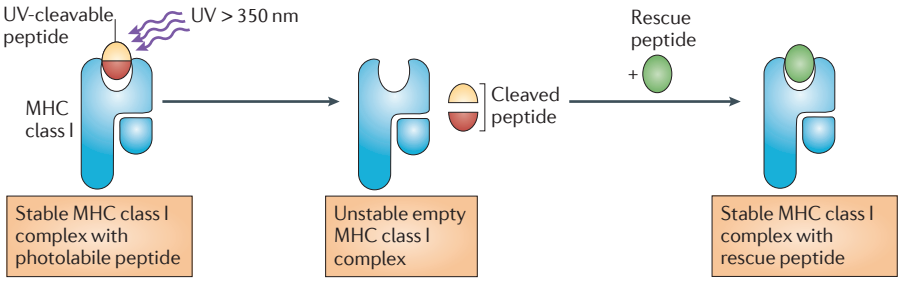


Figure 2 | **UV-mediated peptide-exchange technology.** Biotinylated MHC molecules are produced in complex with an ultraviolet (UV)-sensitive MHC-binding peptide. After UV-mediated cleavage of the photolabile peptide with 350 nm light, which exerts no damage on the rest of the protein, empty MHC molecules will readily bind to a 'rescue peptide'. The MHC molecule bound to this rescue peptide can then be used for peptide–MHC tetramer staining. This UV-mediated peptide exchange reaction can be done on a small scale to allow for the production of a large number of different peptide–MHC tetramers with ease. Image is modified, with permision, from REF. 84 © (2006) Macmillan Publishers Ltd. All rights reserved.

Table 1 | **Types of tetramer, production and applications**

Type of tetramer	Protein production method	Target cells or molecules (or uses)	Refs
Classical MHC molecules			
MHC class I	Bacterial expression followed by refolding	$CD8^+$ αβ T cells	7,85
MHC class II	Bacterial expression followed by refolding, CHO or HEK293 mammalian cell expression, *Drosophila melanogaster* cell expression or expression by insect cells transduced with baculovirus	$CD4^+$ αβ T cells	14, 86–89
Non-classical MHC molecules			
CD1d	Expression by bacteria, *Drosophila melanogaster* cells, insect cells transduced with baculovirus or HEK293T cells transduced with lentivirus	NKT cells	23–25
H2-T10, H2-T22	Bacterial expression followed by refolding	Mouse γδ T cells	26
MICA	Expression by insect cells transduced with baculovirus	Human γδ T cells	27
Non-MHC molecules			
TCR	Various	Used for MHC typing and peptide library screening	28–33
KLRG1		KLRG1 ligands	
NKG2D		NKG2D ligands	
NKG2D ligands		NKG2D-expressing cells	
CCL19		CCR7-expressing cells	

CCL19, CC-chemokine ligand 19; CCR7, CC-chemokine receptor 7; CHO, Chinese hamster ovary; HEK, human embryonic kidney; KLRG1, killer cell lectin-like receptor subfamily G, member 1; MICA, MHC class I polypeptide-related sequence A; NKG2D, natural killer group 2, member D; NKT, natural killer T; TCR, T cell receptor.

number of T cell specificities than the number of fluorophores used in a single sample. In theory, the number of specificities that can be detected using *n* fluorophores equals $2^n - 1$. For example, by dedicating four fluorophores to peptide–MHC tetramer staining, it is possible to detect up to 15 different T cell specificities (FIG. 3). We (E.W.N. and M.M.D.) routinely use this method, alone or in combination with a T cell enrichment procedure (see below), and can detect 15 different T cell specificities by using four fluorophores and up to 63 T cell specificities with six colours[17]. Hadrup *et al.*[18] used this same approach with peptide–MHC tetramers that had been labelled with different quantum dots to detect 25 different T cell specificities.

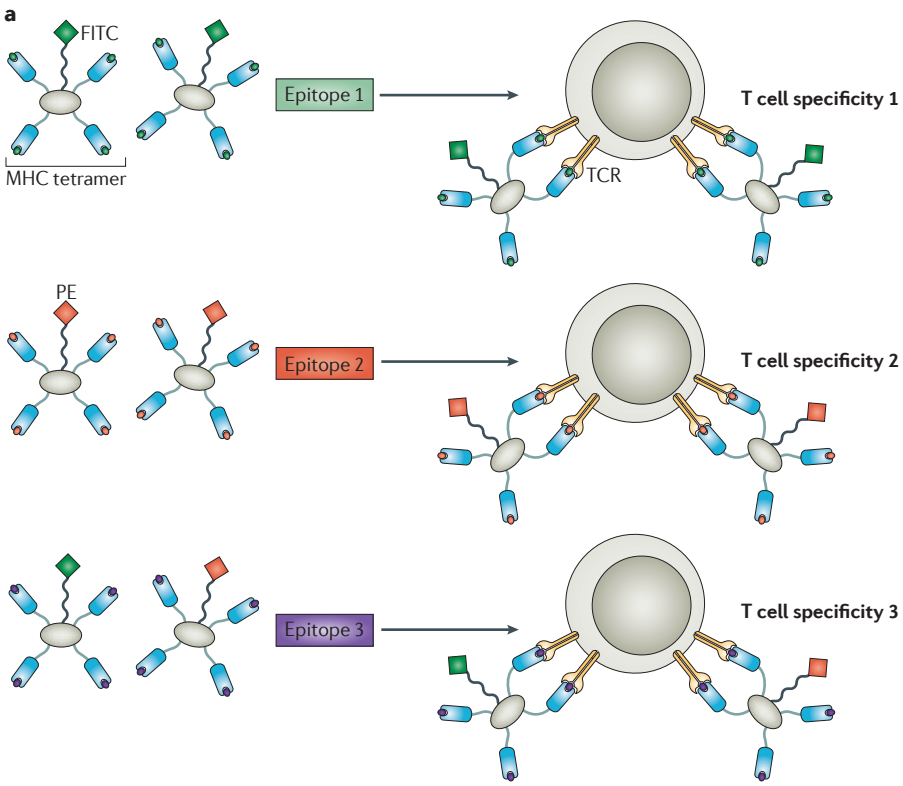
Enriching for labelled T cells. With the exception of T cells that are specific for dominant antigen epitopes or populations that have been expanded during T cell responses, T cells of a given antigen specificity are often extremely rare. This is most pronounced for naive T cells, which do not proliferate before antigen exposure. By an enrichment technique, first developed by Wucherpfennig and colleagues[14], that uses magnetic beads coated with a fluorophore-specific antibody, Moon *et al.*[15] have shown that the detection of rare naive antigen-specific T cells is possible, giving important insights into a previously uncharacterizable but critical part of the repertoire (FIG. 4). We have combined this technique with combinatorial tetramer staining of human samples[17], and have generally achieved a 50–100-fold enrichment of tetramer-bound T cells. This allows a major increase in sensitivity, as previously the detection limit for T cells of a given specificity was at a frequency of approximately 1 in 10,000 T cells. With this enrichment, it is now possible to easily detect antigen-specific T cells with frequencies as low as one in a million. This ability to characterize the naive T cell repertoire also opens up many new possibilities to understand how naive T cells are selected by antigens, as well as to investigate other aspects of their homeostasis[40].

Emerging technologies

CyTOF. Cytometry by time-of-flight mass spectrometry (CyTOF) uses heavy metal ions that are not normally present in biological samples to label cells. CyTOF has the potential to drastically improve cellular analysis by eliminating many of the limitations of fluorescence-based flow cytometry[41–43]. In CyTOF, individual cells are vaporized using an argon plasma torch (at temperatures >7,000 °C), which dissociates all molecules into individual atoms, most of which are also ionized. These ions are then focused into a mass spectrometer, which detects exceedingly low numbers of heavy metal ions. In this system the background noise is low and interference between heavy metal ions of different molecular weights is negligible. This eliminates the need to compensate for the overlapping spectra of different fluorophores, which is essential for multicolour fluorescence-based flow cytometry.

The number of heavy metal species that are detectable and amenable to CyTOF is currently over 30, more than double the number of fluorophores that the current state-of-the-art fluorescence flow cytometers can discern, making it a major new option for peptide–MHC tetramer staining applications[43]. Indeed, many more T cell specificities can be probed by CyTOF at the same time, and a large number of channels still remain available for the detection of phenotypic markers (E.W.N., N. Sigal, S. C. Bendall, G. P. Nolan and M.M.D., manuscript in preparation). Furthermore, the use of heavy metal labels is well suited for combinatorial tetramer staining because they lack the confounding noise that is associated with the highly overlapping fluorophores that we currently use for this approach. In theory, the use of 10 out of the 30 or more isotope channels to detect peptide–MHC tetramers could allow the detection of more than 1,000 different T cell specificities. More realistically, depending on the signal strength and the noise, if each T cell specificity is stained simultaneously with only three metals, then over 100 different specificities might be discernable using ten tetramer channels.

In situ *staining with peptide–MHC tetramers.* Flow cytometry has been the most common experimental application of peptide–MHC tetramers, but other applications that have recently been introduced may have an increasing impact on future research. Several groups have developed *in situ* peptide–MHC tetramer staining methods for tissues[44,45]. These techniques allow for the identification of the relative locations of antigen-specific T cells and antigen-expressing cells (such as tumour cells[46] or cells infected with microorganisms[47–49]), as well as for the observation of TCR polarization during immunological synapse formation *in vivo*[49]. Going one step further, Moore and colleagues attached peptide–MHC complexes to a



b

Fluorophore	TCR specificity						
	1	2	3	4	5	6	7
FITC	+	–	–	+	+	–	+
PE	–	+	–	+	–	+	+
APC	–	–	+	–	+	+	+
Detected signal							

Figure 3 | **The combinatorial tetramer staining concept.** We tested how many different fluorophore-conjugated tetramers that had been loaded with the same peptide could stain the same cells and found that up to six commercially available fluorophore-labelled streptavidin reagents could be used simultaneously[17]. Omitting any number of these six fluorophores can be done in 63 ($2^6 - 1$) different ways, so these combinations of fluorophores can, in theory, be used to stain just as many different T cell specificities when all the tetramers are applied to the cells simultaneously. Part **a** illustrates the simplest version of this concept, whereby three T cell receptor (TCR) specificities can be detected using two colours. That is, cells specific for epitope 1 will be stained only with green (fluorescein isothiocyanate (FITC)-labelled) peptide–MHC tetramers, cells specific for epitope 2 will be stained only with red (phycoerythrin (PE)-labelled) peptide–MHC tetramers and cells specific for epitope 3 will stained by both red and green (FITC- and PE-labelled) peptide–MHC tetramers. Part **b** shows how this concept can be expanded to identify seven different epitopes using three colours (FITC-, PE- and allophycocyanin (APC)-labelled tetramers).

superparamagnetic iron oxide nanoparticle, which is an effective magnetic resonance imaging (MRI) contrast reagent. This allowed them to visualize the recruitment of a diabetogenic $CD8^+$ T cell population to the pancreas in real time[50], providing promise for the use of peptide–MHC tetramers in MRI-based diagnostics in humans.

Specific applications

Epitope mapping. As discussed above, being able to produce peptide–MHC tetramers more easily makes their use for epitope screening much more feasible. By using MHC class II molecules tethered to a cleavable CLIP peptide as a substrate for peptide exchange, Kwok and colleagues developed a system of tetramer-guided epitope mapping, which has proved to be very useful in identifying a large number of useful T cell epitopes[16]. Most recently, this approach has been used to nicely identify and characterize T cells specific for both peanut[51] and cow dander[52] allergens, thus enabling insights into the development of allergic responses. Similarly, the ability to make large numbers of peptide–MHC class I tetramers has allowed large-scale epitope screening (FIG. 2), which was first used to identify new influenza epitopes[13]. Since then, this approach has been used on a larger scale to identify new *Chlamydia trachomatis*[21] and gammaherpesvirus[53] epitopes in mice. In both cases, large-scale screening, and the resulting identification of numerous epitopes, has revealed that the breadth of the immune response to these pathogens is greater than previous assessments or estimations had suggested.

Repertoire analysis. Using the magnet-based tetramer enrichment procedure described above (FIG. 4), Moon *et al.* directly compared the frequencies of naive T cells specific for various peptide antigens presented by $I\text{-}A^b$ molecules. They found these frequencies to be in the range of 1 in 50,000 to 1 in 500,000, depending on the epitope. The conservation of this range of frequencies between individuals has been verified and extended to $CD8^+$ T cells in mice[54,55] and in human peripheral blood[56,57]. Several groups have also investigated the relationship between the frequency of naive T cells and the subsequent quality of the immune response to various antigens. The results have shown that precursor frequency is at least partially responsible for the hierarchical dominance of epitope specificities in vesicular stomatitis virus infection in mice[54], lymphocytic choriomeningitis virus infection in mice[55] and hepatitis C viral infection in humans[58]. The frequency of naive T cells, as measured by peptide–MHC tetramer staining, has also been found to influence the quality of tumour-specific T cell responses[59]. Thus, increased tetramer staining sensitivity enabled by the use of enrichment techniques has facilitated the investigation of the function of naive antigen-specific T cells[40].

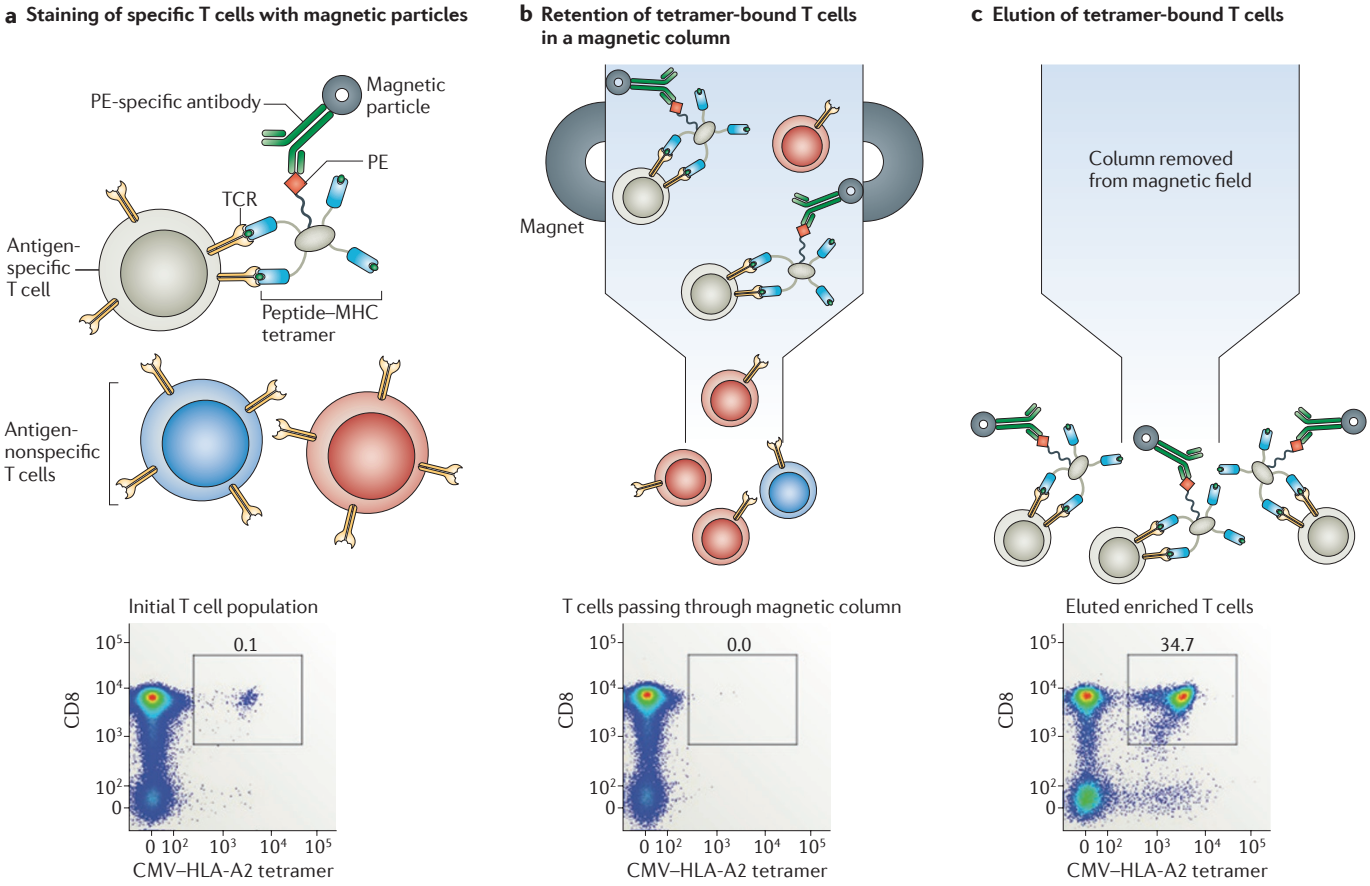


Figure 4 | **Peptide–MHC tetramer enrichment using magnetic particles.** **a** | After staining a sample of CD8⁺ T cells with peptide–MHC tetramers (composed of cytomegalovirus (CMV)–HLA-A2 in this example) in the usual way, the cells are stained with a fluorophore-specific antibody coupled to magnetic iron particles. **b** | The cells are then passed through a magnetized column, in which the iron-labelled cells are trapped by the magnetic field, whereas most of the unlabelled cells are washed away. **c** | The tetramer-bound cells are eluted by removing the column from the magnetic field.

Therapeutic use of peptide–MHC tetramers. Peptide–MHC tetramers have also been used therapeutically. For instance, cytomegalovirus-specific T cells have been enriched to high levels of purity using magnetic bead-based enrichment for use as an adoptive transfer therapy for stem cell transplant patients[60]. In this case, the T cells maintained proliferative capacity following tetramer-mediated enrichment; however, methods using reversible tetramer staining may further improve the functional capacity of these selected cells[61]. Furthermore, several groups have developed tetramer variants — either radiolabelled or coupled to a toxin such as saporin — that can be injected into live mice to modulate or even deplete specific T cell populations[62–66]. For example, toxin-coupled peptide–MHC tetramers were used to ablate T cells specific for various pancreatic epitopes, which demonstrated that a specific tetramer can significantly delay the onset of disease in the NOD (non-obese diabetic) mouse model of type I diabetes[67].

Vaccines for infectious diseases. Many of the most effective vaccines rely on the production of neutralizing antibodies. Therefore, measuring serum antibody levels is a simple and convenient way to assess vaccine efficacy[68]. However, even after immunization with influenza virus vaccines, antibody levels are not always detectable in the serum. Moreover, a vaccine's ability to elicit an effective CD8⁺ T cell response must also be assessed (reviewed in REF. 69). Peptide–MHC tetramers provide an ideal means to characterize the T cells that respond to a vaccine, and they have been used to test T cell responses in many vaccine systems, including influenza[70], yellow fever[71], tuberculosis[72], HIV/SIV[73] and a large number of cancer vaccine trials[74] (discussed below). The ability to detect T cells specific for large numbers of epitopes by the means discussed here should enable accurate analysis of the parameters of a given T cell response, and this is important for understanding the control of rapidly mutating viruses, such as HIV[75].

Cancer immunotherapy. Peptide–MHC tetramers have been widely used to assess CD8⁺ T cell responses to cancer vaccines and other immunotherapies, particularly in patients with melanoma and chronic myeloid leukaemia[12,76–79]. This has allowed researchers to look for correlations between the induced T cell responses and the clinical outcome. In the future, the ability to analyse additional T cell specificities and phenotypic markers will increase our potential to identify the most relevant immune correlates of a course of treatment. It will also be very important to standardize tetramer staining methods, analysis protocols, validation and data sharing. Several large cooperative efforts to achieve these goals are underway[80,81].

Opportunities and challenges

The development of high-throughput methods for tetramer production and of multiplexed analysis — together with new efforts, such as those spearheaded by the NIH epitope-mapping project (which is often

aided by tetramer technology itself[82]) and the associated Immune Epitope Database — will allow investigators to efficiently apply tetramer technology to problems of their particular interest. Biologically important T cell populations that were previously inaccessible owing to their low frequency (such as those specific for allergens) are becoming accessible to analysis through tetramer-based enrichment techniques. In parallel, developments in multiplexed quantitative PCR approaches that allow for the analysis of dozens of transcripts obtained from individual cells using microfluidics devices[83] promise to provide immunologists with a deeper understanding of T cell responses using *ex vivo* analyses.

As in many areas of modern biology, the challenge will be to integrate in a useful way the large volume of information that can be generated by the application of tetramer technology. These data could then contribute to the development of new vaccines for microbial pathogens and tumours, and of modulatory immunotherapies that target autoimmune and hypersensitivity reactions.

Conclusion

Although peptide–MHC multimers have been very useful over the 15 years since their introduction, many improvements in just the past few years have greatly increased their scope, as well as their ease of manufacture and use, and further improvements are visible on the horizon. Thus, users and potential users of these reagents should consider how these new capabilities might be useful to their own research.

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Competing interests statement
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FURTHER INFORMATION
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VIEWPOINT

Plasmacytoid dendritic cells: one-trick ponies or workhorses of the immune system?

Boris Reizis, Marco Colonna, Giorgio Trinchieri, Franck Barrat and Michel Gilliet

Abstract | Plasmacytoid dendritic cells (pDCs) were first described as interferon-producing cells and, for many years, their overlapping characteristics with both lymphocytes and classical dendritic cells (cDCs) created confusion over their exact ontogeny. In this Viewpoint article, *Nature Reviews Immunology* asks five leaders in the field to discuss their thoughts on the development and functions of pDCs — do these cells serve mainly as a major source of type I interferons or do they also make other important contributions to immune responses?

Q How closely related are plasmacytoid dendritic cells (pDCs) to classical dendritic cell (cDC) subsets? Is the name pDC a misnomer?

Boris Reizis. As pointed out by Soumelis and Liu, 'plasmacytoid dendritic' is indeed a misnomer in the strict sense, as it refers to two mutually exclusive cell morphologies[1]. However, I think the name is appropriate in a more general sense, as it reflects the unique dual nature of this cell type. Indeed, pDCs share key features with cDCs, including common progenitors, dependence on the cytokine FMS-related tyrosine kinase 3 ligand (FLT3L) and constitutive expression of its receptor (FLT3), a related global gene expression profile and supreme pathogen-sensing capacity. Moreover, a distinct cDC subset that is closely related