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Missing: A diagnostic technique to enumerate antigen-specific T cells

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Abstract

T lymphocytes are responsible for immune responses against pathogens, immune surveillance against cancer and maintenance of tolerance to self. While techniques available to detect antigen-specific T cells have been well described, there is a missing technique in our repertoire. While fluorescent multimers can be used for limited research applications, there is no existing technique suitable for detection of antigen-specific T cells in a diagnostic setting. The absence of such a technology has inhibited the search for “correlates of protection” against infectious, autoimmune or malignant disease. This critical review of existing methods will highlight the limitations of the data on which our current understanding of the immune system is based, in an effort to stimulate development of improved techniques.

Keywords

Method; Interferon gamma; T cell; T lymphocyte; Immune; Tuberculosis; HIV; Cancer; Tetramer; HLA

1. Introduction

T lymphocytes, together with antibody responses, are responsible for immune responses against pathogens. Also, T lymphocytes maintain tolerance to self antigens and aberrant responses may result in autoimmune disease or malignancy. Much has been written about techniques available to detect antigen-specific T lymphocytes (T cells). What is not usually mentioned is the *absence* of any technique applicable to the clinical setting. This vacuum immediately affects every clinician or scientist researching infectious, autoimmune or malignant disease; but is not often appreciated by non-immunologists. In the current search for biomarkers for malignant and infectious diseases and attempts to develop tumour vaccines, it is vital to draw attention to this missing technology and to stimulate new ideas for its realisation. It is also important to highlight the paucity of data on which our current understanding of T cell mediated conditions is based.

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Conflict of interest: The author holds a provisional patent on a novel method for detection of antigen specific T lymphocytes.

2. Antigen-specific T lymphocytes

Each T lymphocyte has a T cell receptor with a unique structure as a result of V(D)J recombination. This unique structure allows each T cell to respond to a unique peptide fragment presented via a MHC (Major Histocompatibility Complex, in humans known as HLA for Human Leucocyte Antigen) molecule [1–3]. Following stimulation of a naïve T cell, the T cell undergoes proliferation to multiple daughter cells and differentiation to become an effector or memory T cell clone. This process is analogous to a naïve B cell becoming stimulated via its B cell receptor and differentiating into a clone of high-affinity, class-switched (IgG or IgA-producing) plasma cells or memory B cells.

3. Antigen-specific antibodies

Measurement of antibody classes (IgM, IgG, IgA) against particular antigens has become the mainstay of diagnosis for many clinical conditions. The presence of IgG, rather than IgM, to a particular infectious agent is taken as a sign of prior exposure of B lymphocytes to the organism or antigen, and the antibody titre often reflects the time course of the infection. Examples include presence of IgG to Hepatitis B core antigen as a marker of prior natural Hepatitis B infection [4,5], or the presence of IgG to Rubella to indicate Rubella immunity in pregnant women [6]. Antibodies may be employed to diagnose autoimmune diseases, such as the presence of anti-acetylcholine antibodies to diagnose myasthenia gravis or the presence of anti-tissue transglutaminase antibodies to aid diagnosis of coeliac disease [7,8].

4. Detection of antigen-specific T cells in comparison with detection of antigen-specific antibodies

While the presence of specific antibodies has become a routine diagnostic tool, detection of antigen-specific T cells remains limited largely to research applications. Tuberculosis (TB) diagnosis is a notable exception, with use of ELISpot technology (T-SPOT.TB, Oxford Immunotec) and Tcell cytokine production (QuantiFERON-TB Gold, Cellestis) showing utility in certain settings [9]. The paucity of diagnostic applications for detection of antigen-specific T cells is likely due to technical limitations hampering current detection methodologies.

Detection of antigen-specific antibodies is relatively simple. Enzyme immunoassays involve immobilisation of antigen on a solid support (ELISA well, bead, strip etc.) and addition of patient serum. Antigen-specific antibody in the patient serum binds to the immobilised antigen. Any excess unbound antibody is washed off and then bound antibody is detected by a labelled secondary anti-human antibody in a “sandwich” format. The label may be an enzyme which enacts a colour or luminescence change with addition of substrate. The amount or rate of colour change is proportional to amount of antigen-specific antibody in the patient sample. The same principles, however, do not apply to detection of antigen-specific T cells, as T cells will not bind directly to their cognate (corresponding) peptide antigen even if the antigen is immobilised on a solid support. The T cell receptor binds only to its cognate peptide presented in conjunction with a self-MHC molecule (Fig. 1). To immobilise the antigen on a solid support would entail immobilisation of peptide in conjunction with MHC molecule, and would be applicable only to patients having an identical HLA molecule. As HLA molecules are encoded by the most polymorphic human gene locus known, with approximately 300 HLA-A, 600 HLA-B and 100 HLA-C alleles already identified, [10], any one peptide–MHC complex can bind to T cells from very few individuals. Therefore, this approach is not an attractive diagnostic prospect.

5. Shortfalls of existing methods for detection of antigen-specific T cells

The principle of a known HLA molecule presenting a known peptide forms the basis of a highly informative research technique known as multimer staining. This encompasses tetramers (available from National Institute of Health tetramer facility and Beckman Coulter), Pro5@pentamers (ProImmune), Ultimers™ (ProImmune) and MHC Dextramers™ (Immudex)). Multimers are fluorescently tagged MHC–peptide complexes that allow flow cytometric detection of T cells harbouring a cognate T cell receptor [11,12]. The MHC–peptide complex is multimerised to increase binding avidity for the T cell receptor and increase duration of the multimer-T-cell-receptor binding [13]. Multimer staining can be optimised to detect antigen specific T cells at very low frequencies [14–16]. Multimer studies have shown that frequencies of antigen specific CD8⁺ T cells are decreased during active TB infection in comparison with latent infection and return to normal after treatment [17]. They have also shown numbers of cytomegalovirus specific T cells post-haemopoietic stem cell transplant can be used to monitor risk of CMV disease [18]. Tetramer studies have also shown differences in antigen-specific T cells in allergic compared with non-allergic individuals [19]. These studies illustrate the importance of monitoring frequencies of antigen specific T cells in infectious and immune-mediated disease. These studies have however, been limited to patients of selected HLA types.

It is possible to develop an array of MHC–peptide complexes from one or multiple MHC types [20–22]. The cost of manufacture of these multimers in an array format, however, is currently prohibitive. Also, as peptide fragments are generated by proteasomal cleavage of protein molecules into multiple small (8–18 amino acid) fragments, the array of possible antigenic peptides to test for any one disease is virtually infinite. Some groups have devised innovative microarray formats of MHC–peptide complexes coupled with cytokine capture antibodies to detect antigen-specific T cells, with exciting results [23,24]. These approaches deserve further study, as our current repertoire of immunological techniques relies largely on detection of antigen-specific T cell *function* (cytokine secretion or proliferation) rather than enumeration of raw frequency of T cells with particular receptor specificity.

Common techniques used to detect antigen-specific T cells include intracellular cytokine staining, T cell proliferation assays, cytokine immunoassays and cytokine ELISpot assays. These techniques have been reviewed elsewhere [12,25–27, Maecker, 2010]. As a group, they detect function of antigen specific T cells after stimulation with cognate antigen. For example, they will detect gamma interferon production or proliferation of a T cell that has been successfully stimulated through its T cell receptor. They would not however be able to detect whether a proportion of antigen specific T cells is dysfunctional [22,28]. That is, if the cause of a disease is that all the T cells with affinity for the pathogen become dysfunctional, current function-based methods will not be able to differentiate this anergic state from the state of never having any T cells with the correct specificity at all. Functional information is useful if it is expressed in reference to the denominator of total antigen-specific T cells, that is, it would be helpful to know that of 100 tumour specific lymphocytes in a patient with progressive cancer, 17 might be functional. This could be compared with 17 functional cells in a total of 20 total tumour specific lymphocytes in a patient whose tumour is non-progressive. If one evaluates function only, the progressor and non-progressor have the same frequency (17) of functional tumour-specific T cells. The denominator of total frequency of T cells with a particular T cell receptor is missing from the equation and would be analogous to detection of antibodies with a particular function only, for example only blocking antibodies or stimulating antibodies.

An alternative strategy has been to analyse the genes of the T cell receptor via spectratyping or other methods [29–32]. Spectratyping (Immunoscope®) is based on PCR of the T cell

receptor variable gene in T cells from peripheral blood and electrophoretic separation of the PCR products to produce a Gaussian shaped distribution of fragment lengths [33,34]. This approach can be used to find a clone of T cells from amidst a sea of random T cells [30,31,35]. Its limitation, however, is that it gives no idea of the identity of the cognate antigen to which the T cell clone is directed.

6. Relevance of antigen-specific T cells to human disease

There is evidence that many diseases are caused by pathogenic T cells, either CD4⁺ helper T cells or CD8⁺ cytotoxic T cells. This relationship is best exemplified by the MHC association noted in many diseases. An example is the presence of HLA-B*27 in 90% of cases of ankylosing spondylitis [36] or the presence of HLA-DQ2 or HLA-DQ8 [37] with coeliac disease. Despite the HLA specificity, the presence of the HLA type alone is insufficient to confer disease, with only 1.3% of HLA-B*27 positive individuals developing ankylosing spondylitis or 4% of DQ2 individuals developing coeliac disease [37]. As the function of MHCI is to bind the CD8 molecule and present peptides to CD8⁺ T cells and the function of MHCII is to bind the CD4 molecule and present peptides to CD4⁺ cells, the association of HLA polymorphisms with disease is likely due to T cells of particular specificities. Without a laboratory technique with which to analyse the frequency of the antigen-specific T cells, the role of the T cells in pathogenesis cannot be explored.

Even in conditions traditionally regarded as “antibody-mediated” it is possible that antibodies are merely surrogates and not causative agents, despite our reliance on serology in the diagnostic arena. Furthermore, the production of high-affinity antibodies is dependent on T cell help, raising the possibility that even in antibody-mediated autoimmune diseases the initiating abnormality is in autoreactive helper T cells. As an example, the well-characterised autoimmune disease of myasthenia gravis, mediated by antibodies against the acetylcholine receptor, is associated with thymoma and often treated with thymectomy, suggesting a T cell abnormality as being causative. Additionally, 15% percent of cases are seronegative for anti-acetylcholine receptor antibodies and 50% of patients with only ocular signs are seronegative [8]. This scenario is similar in many autoimmune conditions, for example IgA antitissue transglutaminase is the favoured current serological assay for coeliac disease, yet coeliac disease is common in IgA deficient individuals [7,38]. Anti-intrinsic factor antibodies are used as a diagnostic test for pernicious anaemia, but these antibodies are found in only 60% of cases [39]. Many auto-antibodies are found in healthy individuals without causing symptoms, often increasing in frequency with age. These facts should lead us to question whether these and other diseases could be better diagnosed and monitored with tests to detect pathogenic T cell specificities rather than antibody specificities alone.

7. Uncharted territory

Further, the role of T cell *subsets* with any particular specificity has hardly begun to be explored. It would be ideal to be able to quantitate the ratio of Th1 to Th2 to Th17 to T regulatory cells (Tregs) of the same T cell receptor specificity. Crude measurements of total frequencies of regulatory T cells in whole blood without regard to T cell specificity, for example, is unlikely ever to answer questions regarding immunity to one particular pathogen, be it HIV, TB or other [40]. Attempts to measure Tregs (or other T cell subsets) following antigenic stimulation are subject to two disadvantages. The first disadvantage is the method's dependence on *in vitro* recall responses. This implies the method is unable to detect naïve antigen-specific T cells (analogous to being able to detect IgG without differentiation from IgM). This results in the inability to distinguish current from prior infection, for example in the case of TB interferon gamma release assays [9]. The second disadvantage of commonly used techniques is dependence on investigator bias regarding

which predetermined output is to be recorded, for example detection of gamma interferon or another cytokine of interest, cell proliferation or cell degranulation [41].

8. What is missing

What is required is development of a method that quantitates antigen-specific T cells without a re-stimulation step and is applicable to the general population, without pre-selection of HLA type (see Table 1). Without this method, the search for “correlates of protection” for tumour or infectious disease vaccines is limited to seeing what is there, rather than what is not. While we can look for T cell cytokine production or T cell proliferative responses to tumour antigens, this number has no denominator, that is, we cannot say *what proportion of total antigen specific T cells* has these functions. While the technique may take some time coming, it is important to keep in mind both what is not known as well as what is known, and which questions have not been asked at all. The question of how many antigen-specific T cells are present in infectious, autoimmune disease or malignancy, has in fact, not been asked at all, except in a handful of multimer studies in a handful of HLA types.

9. Conclusion

The purpose of this review is to frame the challenge – to develop novel methodologies for enumeration of antigen-specific T cells, without *ex vivo* stimulation, without reliance on T cell function as readout and applicable to any HLA type. Without such a technique, it is unsurprising that immuno-pathogenesis of autoimmune disease remains mysterious or correlates of protection of HIV and TB vaccines elude us. Data from existing methods should be interpreted with caution, as available data represents only a portion of the complete picture.

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References

1. Davis MM, Chien YH, Gascoigne NR, Hedrick SM. A murine T cell receptor gene complex: isolation, structure and rearrangement. *Immunol Rev.* 1984; 81:235–58. [PubMed: 6096259]
2. Sim GK, Yague J, Nelson J, et al. Primary structure of human T-cell receptor alpha-chain. *Nature.* 1984; 312:771–5. [PubMed: 6440030]
3. Zinkernagel RM, Doherty PC. The concept that surveillance of self is mediated via the same set of genes that determines recognition of allogenic cells. *Cold Spring Harb Symp Quant Biol.* 1977; 41(Pt.2):505–10. [PubMed: 302174]
4. Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep.* 2005; 54:1–31. [PubMed: 16371945]
5. Rubella vaccines: WHO position paper. *Wkly Epidemiol Rec.* 2011; 86:301–16. [PubMed: 21766537]
6. Watson JC, Hadler SC, Dykewicz CA, Reef S, Phillips L. Measles, mumps, and rubella – vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep.* 1998; 47:1–57. [PubMed: 9639369]
7. Hill PG, McMillan SA. Anti-tissue transglutaminase antibodies and their role in the investigation of coeliac disease. *Ann Clin Biochem.* 2006; 43:105–17. [PubMed: 16536912]

8. Meriggioli MN. Myasthenia gravis with anti-acetylcholine receptor antibodies. *Front Neurol Neurosci.* 2009; 26:94–108. [PubMed: 19349707]
9. Pai M. Spectrum of latent tuberculosis—existing tests cannot resolve the underlying phenotypes. *Nat Rev Microbiol.* 2010; 8:242. author reply 242. [PubMed: 20084059]
10. Jin P, Wang E. Polymorphism in clinical immunology – from HLA typing to immunogenetic profiling. *J Transl Med.* 2003; 1:8. [PubMed: 14624696]
11. Altman JD, Moss PA, Goulder PJ, et al. Phenotypic analysis of antigen-specific T lymphocytes. *Science.* 1996; 274:94–6. [PubMed: 8810254]
12. Chattopadhyay PK, Melenhorst JJ, Ladell K, et al. Techniques to improve the direct ex vivo detection of low frequency antigen-specific CD8+ T cells with peptide-major histocompatibility complex class I tetramers. *Cytometry A.* 2008; 73:1001–9. [PubMed: 18836993]
13. Vollers SS, Stern LJ. Class II major histocompatibility complex tetramer staining: progress, problems, and prospects. *Immunology.* 2008; 123:305–13. [PubMed: 18251991]
14. Scriba TJ, Purbhoo M, Day CL, et al. Ultrasensitive detection and phenotyping of CD4+ T cells with optimized HLA class II tetramer staining. *J Immunol.* 2005; 175:6334–43. [PubMed: 16272285]
15. Guillaume P, Dojcinovic D, Luescher IF. Soluble MHC–peptide complexes: tools for the monitoring of T cell responses in clinical trials and basic research. *Cancer Immun.* 2009; 9:7. [PubMed: 19777993]
16. Cameron TO, Norris PJ, Patel A, et al. Labeling antigen-specific CD4(+) T cells with class II MHC oligomers. *J Immunol Methods.* 2002; 268:51–69. [PubMed: 12213343]
17. Caccamo N, Guggino G, Meraviglia S, et al. Analysis of *Mycobacterium tuberculosis*-specific CD8 T-cells in patients with active tuberculosis and in individuals with latent infection. *PLoS One.* 2009; 4:e5528. [PubMed: 19436760]
18. Gratama JW, Boeckh M, Nakamura R, et al. Immune monitoring with iTAg MHC Tetramers for prediction of recurrent or persistent cytomegalovirus infection or disease in allogeneic hematopoietic stem cell transplant recipients: a prospective multicenter study. *Blood.* 2010; 116:1655–62. [PubMed: 20508161]
19. Macaubas C, Wahlstrom J, Galvao da Silva AP, et al. Allergen-specific MHC class II tetramer+ cells are detectable in allergic, but not in nonallergic, individuals. *J Immunol.* 2006; 176:5069–77. [PubMed: 16585604]
20. Soen Y, Chen DS, Kraft DL, Davis MM, Brown PO. Detection and characterization of cellular immune responses using peptide–MHC microarrays. *PLoS Biol.* 2003; 1:E65. [PubMed: 14691537]
21. Gaseitsiwe S, Valentini D, Ahmed R, et al. Major histocompatibility complex class II molecule–human immunodeficiency virus peptide analysis using a microarray chip. *Clin Vaccine Immunol.* 2009; 16:567–73. [PubMed: 19225081]
22. Hadrup SR, Schumacher TN. MHC-based detection of antigen-specific CD8+ T cell responses. *Cancer Immunol Immunother.* 2010; 59:1425–33. [PubMed: 20177676]
23. Stone JD, Demkowicz WE Jr, Stern LJ. HLA-restricted epitope identification and detection of functional T cell responses by using MHC–peptide and costimulatory microarrays. *Proc Natl Acad Sci U S A.* 2005; 102:3744–9. [PubMed: 15728728]
24. Chen DS, Soen Y, Stuge TB, et al. Marked differences in human melanoma antigen-specific T cell responsiveness after vaccination using a functional microarray. *PLoS Med.* 2005; 2:e265. [PubMed: 16162034]
25. Hanekom WA, Hughes J, Mavinkurve M, et al. Novel application of a whole blood intracellular cytokine detection assay to quantitate specific T-cell frequency in field studies. *J Immunol Methods.* 2004; 291:185–95. [PubMed: 15345316]
26. Gratama JW, Kern F, Manca F, Roederer M. Measuring antigen-specific immune responses, 2008 update. *Cytometry A.* 2008; 73:971–4. [PubMed: 18942721]
27. Bercovici N, Duffour MT, Agrawal S, Salcedo M, Abastado JP. New methods for assessing T-cell responses. *Clin Diagn Lab Immunol.* 2000; 7:859–64. [PubMed: 11063487]

28. Ueno T, Tomiyama H, Fujiwara M, Oka S, Takiguchi M. Functionally impaired HIV-specific CD8 T cells show high affinity TCR–ligand interactions. *J Immunol.* 2004; 173:5451–7. [PubMed: 15494492]
29. Du G, Qiu L, Shen L, et al. Combined megaplex TCR isolation and SMART-based real-time quantitation methods for quantitating antigen-specific T cell clones in mycobacterial infection. *J Immunol Methods.* 2006; 308:19–35. [PubMed: 16403511]
30. McLean-Tooke A, Barge D, Spickett GP, Gennery AR. T cell receptor Vbeta repertoire of T lymphocytes and T regulatory cells by flow cytometric analysis in healthy children. *Clin Exp Immunol.* 2008; 151:190–8. [PubMed: 17983445]
31. Lim A, Baron V, Ferradini L, Bonneville M, Kourilsky P, Pannetier C. Combination of MHC–peptide multimer-based T cell sorting with the Immunoscope permits sensitive ex vivo quantitation and follow-up of human CD8+ T cell immune responses. *J Immunol Methods.* 2002; 261:177–94. [PubMed: 11861076]
32. Bonarius HP, Baas F, Remmerswaal EB, et al. Monitoring the T-cell receptor repertoire at single-clone resolution. *PLoS One.* 2006; 1:e55. [PubMed: 17183685]
33. Kim G, Tanuma N, Kojima T, et al. CDR3 size spectratyping and sequencing of spectratype-derived TCR of spinal cord T cells in autoimmune encephalomyelitis. *J Immunol.* 1998; 160:509–13. [PubMed: 9552010]
34. Tiev KP, Abriol J, Burland MC, Antonelli D, et al. T cell repertoire in patients with stable scleroderma. *Clin Exp Immunol.* 2005; 139:348–54. [PubMed: 15654834]
35. Laplaud DA, Ruiz C, Wiertlewski S, et al. Blood T-cell receptor beta chain transcriptome in multiple sclerosis. Characterization of the T cells with altered CDR3 length distribution. *Brain.* 2004; 127:981–95. [PubMed: 14985265]
36. Taurog JD. The role of HLA-B27 in spondyloarthritis. *J Rheumatol.* 2010; 37:2606–16. [PubMed: 21123333]
37. Tjon JM, Verbeek WH, Kooy-Winkelaar YM, et al. Defective synthesis or association of T-cell receptor chains underlies loss of surface T-cell receptor-CD3 expression in enteropathy-associated T-cell lymphoma. *Blood.* 2008; 112:5103–10. [PubMed: 18815285]
38. Wang N, Shen N, Vyse TJ, et al. Selective IgA deficiency in autoimmune diseases. *Mol Med.* 2011
39. Carmel R. Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race. *Clin Exp Immunol.* 1992; 89:74–7. [PubMed: 1628426]
40. Shafiani S, Tucker-Heard G, Kariyone A, Takatsu K, Urdahl KB. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J Exp Med.* 2010; 207:1409–20. [PubMed: 20547826]
41. Makedonas G, Betts MR. Living in a house of cards: re-evaluating CD8+ T-cell immune correlates against HIV. *Immunol Rev.* 2011; 239:109–24. [PubMed: 21198668]

Biography

Dr. Melinda Suchard is a medical doctor, specialised in clinical pathology, which involves diagnostic haematology, microbiology and clinical chemistry. Dr. Suchard established the Immuno-haematology division of the Department of Molecular Medicine and Haematology, National Health Laboratory Service, which offers flow cytometric diagnostic tests including detection of paroxysmal nocturnal haemoglobinuria, crossmatching for renal transplant, and Luminex-based anti-HLA antibody detection. Her research interests involve the role of regulatory T cells in infectious diseases such as HIV and Tuberculosis. She is currently completing her PhD which involves development of a novel method for detection of antigen specific T lymphocytes. She supervises a number of post-graduate students and lectures medical students, registrars and fellows in wide-ranging clinical fields such as internal medicine, paediatrics, nephrology, haematology and tropical medicine. She runs a 2-week course in Basic and Advanced Immunology for clinicians and scientists at the University of the Witwatersrand. She is a member of the executive committee of the South African

Immunology Society. In 2011 Dr. Suchard received the prestigious Discovery Foundation Academic Fellowship Award.

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Fig. 1. Difference between antigen presentation to T and B lymphocytes

B and T lymphocytes respond to different epitopes of an antigen. B cell epitopes are surface configurations of the antigen, and include protein, polysaccharide or lipid motifs. T cell epitopes are short, contiguous amino acid sequences arising from either surface or internal structures within the antigen that have undergone proteasomal processing within the antigen presenting cell and have been loaded onto self-MHC (HLA) molecules. While there are numerous techniques to enumerate antibodies to a certain antigen, there are few that can count T cells with a particular T cell receptor.

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Table 1
Qualities of existing techniques in comparison with desirable qualities of a novel technique to detect antigen-specific T cells.

	Reliance on <i>in vitro</i> re-stimulation	Reliance on prior knowledge of peptide fragment sequence of antigen	Requirement for prior knowledge of HLA type	Ability to distinguish T cell subsets	Ability look at more than one function simultaneously	Expense (low, intermed, high)
Methods dependent on functional readout						
Intracellular cytokine staining by flow cytometry	Yes	No ^a	No	Yes	Yes	Intermediate
Cytokine immunoassay (including interferon gamma release assays)	Yes	No ^a	No	No ^b	Yes	Intermediate
ELISpot	Yes	No ^a	No	No ^b	Yes	Intermediate
Methods independent of T cell function						
Spectratyping (Immunoscope)	No	No (disadvantage is that corresponding antigen often unknown)	No	No ^b	No	Intermediate
Fluorescent multimer flow cytometry	No, although practically often necessary	Yes	Yes	Yes	Yes	High
MHC-peptide microarrays	No	Yes	Yes	No ^b	Yes (if cytokine capture antibodies included in array [1])	High
Missing method						
Desirable technique	No	No	No	Yes	Yes	Low

^aCan use whole or peptide antigen.
^bUnless pre-sorted.