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Deconvolving heterogeneity in the CD8⁺ T cell response to HIV

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Abstract

Purpose of review—This review will discuss the use of systems biology approaches to dissect the heterogeneity of the HIV-specific CD8⁺ T cell response.

Recent findings—New experimental approaches have allowed complex phenotypes of individual cells present in the human antigen-specific CD8⁺ T cell response to be characterized. Genome-wide measurements of gene expression in antigen-specific T cells have created broad molecular phenotypes of the T cell response to HIV. Pattern recognition algorithms to discover new sub-classes of samples in microarray datasets are becoming increasingly sophisticated. Together, these advances now allow the heterogeneity of the T cell response to HIV to be unraveled.

Summary—The phenotype antigen-specific T cells responding to pathogens like HIV in humans is seen as much “noisier” than in animal models of infection. However, applying new systems biology tools may provide an opportunity to identify the sources of this “noise” as novel, biologically distinct sub-classes of CD8⁺ T cell response to HIV.

Keywords

HIV; heterogeneity; genomics; single-cell

Introduction

During a prototypical immune response to a viral pathogen, antigen-specific CD8⁺ T cells become activated, proliferate and gain effector functions [1]. This initial burst of proliferation yields a large number of effector cells, most of which will die during the contraction phase, leaving a smaller population of antigen-specific cells that form the memory pool. However, the population of antigen-specific CD8⁺ T cells formed in response to a viral infection is highly heterogeneous in terms of function and phenotype [2]. In acute infections, the memory pool includes a mixture of cells with variable longevity and proliferative potential. In chronic infections like HIV, the antigen-specific CD8⁺ T cell population also includes cells that progressively lose cardinal functions such as IL-2 secretion, proliferation potential, and cytotoxicity in a process known as T cell exhaustion [3**]. T cells specific for HIV therefore represent a diverse collection of T cell states with a range of abilities to exert control over the virus. This heterogeneity is reflected in a complex pattern of gene expression that characterizes each of those states. This diversity poses a

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particular challenge for immunologists trying to identify the characteristics of T cells that contribute to a protective immune response to the virus [4].

Tools to capture global sets of complex biological data (such as measurements of DNA sequence, or quantification of RNA transcript, protein or metabolite abundance) have become widely available and commonly used [5]. These tools can reveal how the component processes within a cell contribute to its biological function in a way that is not evident from examining them individually [6*]. In addition, analysis of high-dimensional data is able to unravel heterogeneity present in a collection of biological samples. For instance, in cancer biology, unsupervised analysis of gene expression profiles has revealed novel subtypes of cancer associated with discrete clinical phenotypes and outcomes [7,8,9].

This review will outline how heterogeneity in the CD8⁺ T cell response at the single-cell level arises. Next, we will review new data showing how the heterogeneity of antigen-specific T cells can be characterized. We will outline how the analysis of high-dimensional data can be used to identify discrete sets of biologically related samples. Lastly, we will discuss recent literature that shows how systems biology could be used to dissect heterogeneity in the virus-specific CD8⁺ T cell response in subjects with HIV.

Origins of heterogeneity in the CD8⁺ T cell compartment

The first level of diversity within the CD8⁺ T cell response originates in the sequence of the TCR. Each T cell specific for a given peptide-MHC complex (pMHC) expresses a specific TCR sequence – its clonotype – and the antigen specific response that is found in a pMHC-tetramer⁺ population is composed of multiple clonotypes [10]. Each distinct TCR will recognize the pMHC with a different avidity, and the biophysical characteristics of this interaction lead to cells with different antigen sensitivities [11,12]. However, even when cells expressing genetically identical TCRs are stimulated in vivo the resulting effector and memory pools show marked heterogeneity [13]. Environmental factors such as antigen dose, duration of contact, and a large set of receptor ligand interactions can profoundly alter the resulting pattern of gene expression [1]. Thus a pMHC-tetramer⁺ population of cells represents a collection of diverse T cell states.

Quantifying heterogeneity in human antigen-specific CD8⁺ T cells

The discovery of pMHC-tetramers have provided a critical tool with which to dissect the heterogeneity of the pool of antigen-specific CD8⁺ T cells in humans [14]. Tetramers allow antigen-specific T cells to be identified and isolated agnostic of their surface phenotype, and have been combined with multi parameter flow cytometry to identify the phenotype of T cells responding to viruses [15,16**] and tumors [17]. Several recent technological advances have allowed the analysis of single antigen-specific T cells with many more parameters than previously possible.

Flatz and colleagues analyzed the gene expression profiles of single HIV-specific CD8⁺ T cells elicited by vaccine regimens in a mouse model [18**]. Using a nanofluidic device, the expression of 96 genes was assayed from cDNA generated from individual cells, revealing a complex picture of gene expression in individual cells generated by vaccination. Although conventional flow-cytometric analysis of the CD8⁺ T cells elicited by the vaccines showed overlapping phenotypic and functional profiles, analysis of single cell gene expression revealed marked heterogeneity between the vaccine-induced responses. Novel phenotypes of T cells within each vaccine response could be defined based on the expression of previous uncharacterized combinations of marker genes.

An analogous approach has recently been developed to assay multiple functional phenotypes in single cells measured with a barcoded set of nanowells [19**]. This revealed that melanoma-specific CD8⁺ T cells of a single phenotype (effector memory cells expressing CD45RA) showed striking heterogeneity. The investigators measured 12 different effector cytokines allowing for >4,000 possible unique combinations. However, examination of 10,000 cells yielded only 45 combinations that accounted for the majority of cells. This number of distinct subsets was relatively few, suggesting a non-random assortment of functional states.

As techniques to assay the expression of large numbers of genes expressed by single antigen-specific cells expand, several challenges emerge. First is the difficulty in distinguishing true heritable differentiation states from transient activation phenotypes. Second is the challenge of isolating sufficient numbers of increasingly rare subpopulations of T cells to allow them to be studied. Finally, linking new T cell phenotypes to their ability to confer immunologic protection in individuals with HIV infection becomes a more difficult task as candidate phenotypes multiply.

Linking cell phenotype to clinical phenotype through supervised analysis

Many studies have identified phenotypic markers expressed by HIV-specific CD8⁺ T cells associated with superior viral control. Most of these studies have used a “supervised” analysis in which the expression of candidate CD8⁺ T cell markers are compared, usually by flow cytometry, between cohorts of patients with defined clinical phenotypes. This experimental approach has its roots in studies of animal models of acute and chronic viral infection in which the effects of viral persistence can be readily assessed. Studies in humans with chronic viral infection has led to the discovery of surface markers such as PD-1 and CTLA4 [20,21,22,23] that are closely associated with T cell exhaustion.

Because flow-cytometry is limited in the number of candidate phenotypic markers that can be simultaneously surveyed in antigen-specific T cells, several groups have assayed the gene expression profile of antigen-specific T cells using cDNA microarrays to create broad molecular phenotypes [24,25*,26*,27*]. We studied gene expression profiles of Gag-specific T cells from infected individuals with chronic elevation of viral load in the absence of anti-retroviral therapy (progressors) and compared them to individuals with spontaneous control of viral replication (controllers) [27*] in order to study virus-specific T cells at the extremes of functional competence [28]. We found that the global pattern of gene expression in HIV-specific T cells from progressors compared to controllers was highly similar to that seen in mouse LCMV-specific T cells responding to chronic versus acute viral infection. Moreover, analysis of the genes that distinguish between exhausted CD8⁺ T cells and their more functional counterparts identified a new role for the transcription factor BATF in suppressing proliferation and cytokine secretion, possibly by modulating AP-1 target genes.

A similar transcriptional analysis of antigen-specific T cells in melanoma also found that tumor-specific CD8⁺ T cells from the tumor site (but not from the circulating melanoma-specific CD8⁺ T cell pool) showed significant similarity to the expression profile of exhausted CD8⁺ T cells from the LCMV mouse model [25*]. These studies suggest that the analysis of genome-wide expression profiles can be used to define the broad molecular phenotype of antigen-specific T cells in humans, and may be able to identify novel genes that are both markers and mediators of T cell dysfunction in chronic viral disease or cancer.

Linking cell phenotype to clinical phenotype through class discovery

Unlike genetically homogeneous animal models, the phenotype of the HIV-specific CD8⁺ T cell response in humans shows much more variability. Variability in individual phenotypic

markers could arise from stochastic inter-subject differences in the expression of a single gene by antigen-specific cells. However, given the enormous heterogeneity that arises during CD8⁺ T cell differentiation it is also possible that this variability represents true biological diversity in the CD8⁺ T cell response to HIV. Studying small numbers of genes expressed by antigen-specific T cells may not provide sufficient complexity with which to unequivocally demarcate new types of CD8⁺ T cell responses among this heterogeneous pool. Systems biology has the potential to capture the extent of heterogeneity that exists in the HIV-specific T cell response and discover new subclasses of antigen-specific T cell response.

Subclass discovery using microarray data

Analytic approaches to discover new subclasses of samples based on distinct patterns of gene expression have been extensively used in cancer genomics to identify new categories of tumors types with distinct molecular, prognostic, or drug-response features [7,8,9,29,30]. Clustering approaches share one critical aspect: the analysis is unsupervised. In contrast to supervised approaches in which the biologist assigns samples to specific groups (e.g. progressors vs. elite controllers), unsupervised analytic techniques make no a priori assumptions of how many subclasses of samples exist in a dataset. The goal of unsupervised clustering approaches is to identify new groups of samples that share common patterns of gene expression.

The basic assumptions of clustering approaches are twofold. The first is that samples that belong to distinct biological states will share similar expression patterns across a large numbers of genes. This assumption is supported by increasing data that suggest that differentiation within the hematopoietic system is governed by well-circumscribed patterns of gene expression [31**]. Based on robust similarities in the pattern of sets of genes, the columns (samples) of a hypothetical data table can be grouped together into classes of samples that are putatively similar in important biological characteristics. The second assumption is that genes regulated by similar biological mechanisms will show correlation in expression across a number of samples. Evidence for this assumption comes from rigorously controlled experiments studying the behavior of groups of genes known to be subject to similar regulatory influences [32,33,34]. Thus the rows (genes) of a data table can be grouped together into collections of genes that are likely to be involved in common biological mechanisms [35].

The idea of clustering objects in multi-dimensional space should be familiar to immunologists who analyze flow-cytometric data. Populations of cells that similar share staining patterns can be visualized as clusters in, say, a dot-plot of two surface markers. However more complex machine learning techniques are needed to cluster samples profiled by measurement of genome-wide gene expression. Although a detailed comparison of these mathematical algorithms is beyond the scope of this review, the broad consensus is that although many mathematical approaches have been used to cluster samples based on microarray data none is clearly superior for all applications [36].

Discovering new subgroups of patients

The application of approaches to discover new subclasses of patients characterized by distinct virus-specific T cell responses has yet to be performed. However, some studies have used genome-wide transcriptional profiles from CD8⁺ T cells to discover new subgroups of patients with immunologic diseases. These studies lay the analytic and conceptual groundwork for similar approaches in data from antigen-specific T cells.

McKinney and colleagues studied a cohort of patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), a chronic, severe disease characterized by inflammation of medium-sized and small blood vessels in which CD8⁺ T cells are thought to play a prominent role [37**]. Analysis of gene expression profiles from total CD8⁺ T cells using a clustering technique called multidimensional scaling found a greater degree of heterogeneity between samples than would be expected by chance alone. Two predominant subgroups of patients were identified based on molecularly distinct patterns of CD8⁺ T cells that were highly significantly associated with relapse after initial disease remission and with the number of ‘flares’ of disease severity. Remarkably, the same molecular sub-type of CD8⁺ T cells was also found in an independent dataset of CD8⁺ gene expression profiles from patients with SLE. The molecular subtypes of CD8⁺ T cells in SLE were highly related to those seen in AAV suggesting that similar biological mechanisms were manifest in common patterns of gene expression in CD8⁺ T cells. Analysis of genes that best discriminated between the two sub-classes suggested that genes in the IL7R and TCR pathways were differentially expressed, although no clear biological explanation for the existence of these two patterns of gene expression could be defined.

This study exemplifies the general approach to discovery of sub-classes of samples associated with different clinical outcomes. First, clustering approaches were used to group samples together into distinct “buckets” that are more dissimilar to each other than would be expected by chance alone. Second, the subtypes of samples were tested for association with important clinical parameters, such as disease severity or disease outcome. Next, the robustness of these molecular classes of samples was confirmed in an independent dataset. Finally, the genes that discriminate between these subclasses are interrogated for biological meaning to develop hypotheses regarding the basis for the observed differences in clinical outcome. This framework can be readily applied to analysis of gene expression profiles from antigen-specific T cells in order to discover distinct molecular CD8⁺ T cell responses to chronic viral infection present in sub-groups of subjects. Such studies are currently underway by several groups.

Obstacles to using systems approaches to studying heterogeneity in the T cell response to HIV

Several practical limitations need to be addressed to apply systems biology to subclass discovery in the HIV-specific T cell response. Isolating T cells by pMHC-tetramers is limited by the availability of reagents and only a subset of epitope-specific responses can be studied in this way. However, techniques to identify antigen-specific CD8⁺ T cells based on upregulation of activation markers in response to a brief in vitro stimulation may allow a broader range of epitope-specific T cells to be identified, albeit with the loss of studying the ‘unperturbed’ state of the cell [38].

A second drawback is that cDNA microarrays measure abundance of a large but circumscribed set of transcripts. Newer techniques such as RNA-seq cast a broader net, capturing levels of splice-forms of specific transcripts, at a cost of even greater analytic complexity [39]. Integration of orthogonal measurements of RNA abundance, alternative splicing, microRNA abundance and metabolic profiles may provide an even more granular view of cell state but techniques to identify new subclasses of samples across multiple datasets have only just begun to be developed [40*].

Third, the cost of whole-genome microarrays remains prohibitively expensive for examining the T cell response to HIV in large numbers of patients. However, several mid-density gene expression profiling platforms have been developed that can quantify transcript levels of 100

– 1000 genes at relatively low cost compared to whole-genome microarrays or RNA-sequencing [18**,32,41].

Fourth, the mass-spectrometric detection of isotope labeled antibodies promise to markedly expand the complexity of markers that can be profiled by flow cytometry [42**]. However, the number of antibodies compatible with flow cytometry is orders of magnitude lower than the number of transcripts detected by most expression profiling platforms. Developing tools for genome-scale measurement of transcript abundance in single cells is still a priority [43].

Finally, in cancer genomics, understanding heterogeneity within apparently homogeneous collections of tumors has required systematic approaches to collect and analyze annotated banks of tumors with novel technological platforms [7,9,44,45]. Similar approaches to deconvolve the heterogeneity that exists among T cell responses to HIV are likely to require analogous commitments of resources.

Conclusion

When compared to animal models used to study the development of T cell memory [46] and exhaustion [47], the study of human immune response is often viewed as problematic because variability in the phenotype of antigen-specific T cells between individuals is attributed to stochastic noise. However, this underlying heterogeneity can be seen as an opportunity to isolate the sources of this “noise” that may be due to the presence of biologically distinct, as-yet unrecognized sub-populations of T cells. Applying systems biology tools to this task may therefore provide a new approach to discovering new subtypes of T cell response to HIV.

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Key Points

- Recent studies have revealed marked heterogeneity in the antigen-specific CD8⁺ T cell response to HIV.
- New technological and analytic advances have enabled the molecular phenotype of the CD8⁺ T cell response to HIV be mapped in different individuals at the cell population level and at the single-cell level.
- There is a need to better understand true correlates of protection in order for a vaccine for HIV to succeed.
- New systems biology approaches may provide an opportunity to unravel this heterogeneity in order to identify novel, biologically distinct sub-classes of the CD8⁺ T cell response that highly correlate with effective control of HIV.