Published in final edited form as:

Trends Immunol. 2014 June ; 35(6): 231–232. doi:10.1016/j.it.2014.04.004.

Identifying environmental antigens that activate myelin-specific T cells

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Abstract

Human genetic and environmental factors underlie susceptibility to the T cell-mediated autoimmune disease, Multiple Sclerosis (MS). How the environment influences Multiple Sclerosis disease has been difficult to parse. A recent paper in *Cell* shows that environmental antigens that activate myelin-specific T cells can be identified with unprecedented accuracy.

Mature $\alpha\beta$ T cells orchestrate immunity to viruses and microorganisms following the recognition of pathogen-derived peptides displayed on host-major histocompatibility complex (MHC) and MHC-like molecules. The mature repertoire accomplishes this breadth of coverage by being comprised of individual T cell clonotypes capable of functionally engaging multiple distinct peptide/MHC (pMHC) complexes expressed on the surface of cells. The ability of T cell antigen receptors (TCRs) to be cross-reactive is critical for the mature repertoire of T cells to respond to most, if not all, foreign peptides bound to host MHC proteins [1].

Despite the culling of self-reactive T cells by immune tolerance mechanisms, some auto-reactive T cells are present in the mature T cell repertoire and when activated, induce autoimmunity. To understand the triggers of Multiple Sclerosis (MS), Type 1 Diabetes (T1D) and other autoimmune diseases, a great effort has been put into identifying how T cell self-tolerance breaks down, and into determining the antigen specificity of T cells that infiltrate target organs. In T1D, naïve self-reactive T cells may be directly activated by β -islet cell antigens presented within the draining lymph node of the pancreas [2]. In Multiple Sclerosis and other autoimmune diseases, T cells that are normally ignorant of a tissue-restricted antigen may be primed by infectious agents that carry molecular mimics – pathogen-derived antigens that activate self-reactive T cells through peptide cross-reactivity [3, 4]. A gap in the understanding of autoimmune disease etiology, however, comes from the large number of ‘orphan’ T cells present within the target organ whose antigen specificity is unknown.

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To gain insights into T cell cross-reactivity and its role in priming auto-reactive T cells, early studies identified the critical peptide residues required for the activation of several human myelin basic protein (MBP)-specific T cells [5]. Genome-wide peptide homology searches looking for peptides that carried similar TCR and MHC binding sequence motifs led to the discovery that some MBP-specific T cells can cross-react with cells infected with Epstein Barr Virus [4]. The development of peptide scanning libraries, pooled libraries that collectively express all 20 amino acids at each position of the peptide, allowed for a more high-throughput approach to identifying the central T cell recognition motif of the peptide and allowed peptide mimitopes to be identified without knowing the cognate peptide sequence [6]. However, because cell lines usually express multiple MHC alleles and peptides can often bind MHC class II proteins in different registers, challenges can occur when trying to de-convolute the peptide reactivity matrices generated by soluble peptide scanning. More recently, baculovirus pMHC display libraries have been created which fuse the randomized peptide sequences directly to the MHC protein of interest. This genetic approach eliminated the problems of cells expressing multiple MHC alleles, and allows peptides to be 'locked' within a particular MHC binding register. These approaches have identified peptides that activate human and murine MHC class I- and MHC class II-restricted TCRs [7], and using 'orphan' diabetogenic T cells, identified Chromogranin A as an auto-antigen targeted in T1D [8].

Recently, Birnbaum et al. have developed a yeast displayed random-pMHC libraries to identify environmental antigens that activate MBP-specific T cells [9]. Fluorescently labeled TCR multimers are used to probe the yeast libraries. Yeast that specifically binding the TCR multimers are sorted and high throughput sequencing is used to identify pMHC complexes that bind the TCR of interest (Figure 1). Proof of principal experiments showed that many of the peptide mimitopes shared a few conserved side chains with the parental cognate peptide at critical sites of T cell recognition. Similar to studies analyzing TCR recognition of altered peptide ligands [10], these lynchpin or hot spot amino acids of the peptide were surrounded by modifier positions of the peptide that could enhance or limit TCR recognition. Elegant covariation analysis of peptides that had dissimilar sequences showed that the TCR cross-reactivity was not random, while structural analyses show that TCR CDR3 loop flexibility can allow for cooperative binding effects to occur between the CDR3 loops and different residues of the peptide. In the examples shown, TCR interactions with the P5 residue of the peptide influence TCR interactions with the P8 residue of the peptide. These findings demonstrate that CDR3-peptide cooperative binding effects can allow TCRs to specifically recognize sets of peptides that might otherwise appear to be unrelated.

Inherent in all library screens is a need to cast a wide enough net to identify potential 'hits' and a means to whittle away false positives. The genomic sequencing of viruses and microorganisms allows for an extraordinary number of peptide sequences to be identified. By creating peptide search algorithms that account for the central MBP-TCR recognition motifs, positions of the peptide that can modify TCR binding and cooperative binding effects, Birnbaum et al. used proteome database searches to identify thousands of peptides that could potentially activate two human MBP-specific CD4 T cell clones (Figure 1). Remarkably, when 33 peptides were tested, a 94% success rate was achieved with 10 of the peptides derived from environmental proteins inducing maximum T cell response at ligand

concentrations as low as 100nM. Thus, peptide search algorithms that include information regarding the central peptide recognition motif, modifier positions and cooperative binding effects show an unprecedented level of accuracy in identifying peptides that activate myelin-specific T cells.

Now that myelin-specific T cell activating peptides can be accurately identified from environmental sources, future enhancements to the peptide search algorithms will be needed to predict whether a specific pathogen infection will trigger autoimmune T cell responses. In particular, next-generation search algorithms will have to take into consideration whether peptide cleavage sites are located within the native protein such that the particular peptide is created during protein degradation, how well the peptide binds different MHC molecules, how H2-DM susceptibility affects presentation of the peptide, as well as evaluating aspects of T cell immunodominance. Although constructing search algorithms with these add-on criteria may appear daunting, the flow path employed by Birnbaum et al. provides a critical step in this process.

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Figure 1.

Experimental flow path for identifying environmental antigens that activate myelin-specific T cells. (A) Fully randomized HLA-DR15-peptide libraries are created in yeast, (B) probed with fluorescently-labeled TCR multimers and TCR-bound yeast are sorted and sequenced to identify critical TCR and MHC binding residues. (C) 2nd generation HLA-DR15-peptide libraries are created that incorporate the critical TCR binding (red) and MHC binding (green) residues, stained with TCR multimers, sorted and sequenced, allowing for the characterization of peptide positions that modify TCR recognition (blue). (D) Peptide sequence information from the 2nd generation library screen is used to create peptide search algorithms to identify potential antigens, and candidate antigens are validated.