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Meeting Report: NIH Workshop on the Tuberculosis Immune Epitope Database

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Abstract

The Immune Epitope Database (IEDB), an online resource available at http://immuneepitope.org/, contains data on T cell and B cells epitopes of multiple pathogens, including *M. tuberculosis*. A workshop held in June, 2007 reviewed the existing database, discussed the utility of reference sets of epitopes, and identified knowledge gaps pertaining to epitopes and immune responses in tuberculosis.

Keywords

tuberculosis; immunity; epitope; lymphocyte; HLA; antibody

Introduction

The National Institutes of Health, National Institute of Allergy and Infectious Diseases, sponsored a workshop in Potomac, Maryland on June 26, 2007, to review the database and

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analysis tools created by the Immune Epitope Database and Analysis Resource (IEDB) pertaining to *M. tuberculosis* and other mycobacteria. At the workshop, the authors and other experts on immune responses to mycobacteria reviewed the database and discussed the value of the format and the curated data on mycobacterial epitopes, and discussed the biological relevance, potential, and shortcomings of the current information.

As recently reviewed in depth[1, 2], adaptive immune responses to *M. tuberculosis* and other mycobacteria involve $CD4^+$ and $CD8^+$ T lymphocytes, whose peptide antigens are presented by MHC (HLA in human) class II and class I, respectively. In addition, recent work has revealed that T lymphocytes isolated from humans infected with *M. tuberculosis* can recognize specific lipids, phospholipids, and glycolipids presented by nonpolymorphic CD1a, CD1b, or CD1c molecules on dendritic cells (reviewed in[3]). Moreover, while antibodies are widely thought to be of little, if any, protective value in tuberculosis, antibody responses to mycobacterial antigens are common, and may have diagnostic value[4].

The identification and characterization of specific mycobacterial antigens and epitopes recognized by B and T lymphocytes has considerable value for research, as well as demonstrated practical value in the diagnosis of latent tuberculosis. For example, as a result of research studies on the immune response to *M. tuberculosis*, identification of commonly-recognized antigens and their epitopes has led to development of a recombinant BCG vaccine that overexpresses antigen 85B[5] and is currently being studied in clinical trials in humans. Investigation of the $CD4^+$ T cell epitopes contained in Ag85B identified peptide-25 and the subsequent preparation of mice with a transgenic T cell antigen receptor (TCR) specific for peptide-25 and the murine class II allele $I\text{-}A^b$, has proven valuable in studies of the adaptive immune response to *M. tuberculosis*[6–9]. In addition, identification of specific *M. tuberculosis* epitopes recognized by $CD4^+$ and $CD8^+$ T lymphocytes during murine infection has permitted study of the frequency, trafficking, differentiation, and fate of antigen-specific T lymphocyte populations during infection[10, 11]. Knowledge of mycobacterial epitopes recognized by $CD4^+$ and $CD8^+$ T lymphocytes from humans infected with *M. tuberculosis* is essential for preparation of peptide-loaded HLA (class I and II) tetramers for study of the frequency and phenotypes of antigen-specific T lymphocytes in humans with latent or active tuberculosis, and in vaccine trials. Moreover, characterization of antigens (and their epitopes) specific for *M. tuberculosis*, and not present in BCG, has already guided the development of improved interferon gamma-release assays that distinguish between immune responses due to BCG vaccination and those due to latent tuberculosis[12]. It is widely hoped that these efforts will also provide the foundation for development of tuberculosis vaccines with improved efficacy against pulmonary tuberculosis in adults. With this background, the Immune Epitope Database and Analysis Resource (IEDB), with the support of the U.S. National Institutes of Allergy and Infectious Diseases (NIAID), initiated a project to collect, curate, and provide ready access to the existing knowledge of epitopes derived from *M. tuberculosis* and other mycobacteria.

The Immune Epitope Database

The Immune Epitope Database (IEDB), a continuously-updated online resource available at http://immuneepitope.org/, contains data on T cell and B cells epitopes obtained from published studies, as well as from direct data submissions, with a focus on NIAID Category A, B and C Priority Pathogens and emerging/re-emerging infectious diseases (http://www3.niaid.nih.gov/research/topics/emerging/list.htm), including *M. tuberculosis*. In order to provide data on epitopes and not all immunogenic molecules, only molecular structures with less than 50 amino acid residues or molecular weights less than 5000 Daltons are included. With regard to TB epitopes, the IEDB currently contains data derived from 296 published papers on 1,377 unique mycobacterial epitopes, of which 1,114 are recognized by T cells and

357 by B cells (94 of the epitopes are recognized by both T and B cells). Of these mycobacterial epitopes, 1,343 are peptides and 34 are non-peptidic. Fourteen of the non-peptidic epitopes are recognized by T cells, and include 4 glycolipids, 2 carbohydrates, 1 lipopeptide, and 7 are other small organic molecules; all of these epitopes are restricted by nonclassical MHC class I molecules. The 20 non-peptidic B cell epitopes include 15 carbohydrates, 3 fatty acids, 1 glycolipid, and 1 other small organic molecule.

Sixty-five percent of the defined mycobacterial epitopes are derived from as few as 30 open reading frames (ORFs). For example, 924 epitopes are derived from 270 of the ~4,000 ORFs in the *M. tuberculosis* genome. Rather than indicating that the adaptive immune response in tuberculosis is narrowly focused on a paucity of potential epitopes, the existing knowledge is likely to be a function of the limited studies and technology that have been applied to epitope characterization to date.

In addition to listing epitopes and their sequences or other structural features, the IEDB provides information (when available) on: protein topology and function, for peptide epitopes; the T cell subsets ($CD4^+$, $CD8^+$, or NKT) that recognize the epitopes (either determined directly, or inferred from MHC restriction data); the MHC restriction element (determined directly, or inferred from the purified T cell subtype used to assess responses), and if available, information in the restricting MHC/HLA alleles; information is also included on the T cell assays used in analysis of the epitopes (cytotoxicity, proliferation, secretion of IFNγ, IL-2, IL-4, IL-5, IL-10, or TGFβ, or in vivo delayed-type hypersensitivity). When available for a given epitope, the disease state of the subject(s) whose cells or serum were used to define the epitope is specified; and when possible (rare in human studies, but common for experimental animals), the strain of *M. tuberculosis* with which the experimental subject was infected is specified. Additional data that are included, when available, are: the host range of immune responses to a given epitope; binding data for MHC molecules, including allele data when it exists; whether the epitope confers protection in experimental infections; and the conservancy or uniqueness of the epitope for the species or strain of mycobacterium.

The criteria for inclusion of epitopes in the IEDB are intentionally broad, in order to provide information that is as comprehensive as possible. However, a reference set of epitopes would be useful for the research community for use in ongoing and future studies. Therefore, the IEDB has applied the criteria of detection by direct ex vivo assay (i.e., without in vitro restimulation or amplification) and use of standardized assays (intracellular cytokine staining, ELISPOT, and/or proliferation for T cell responses; ELISA or antigen competition of antibody binding for B cell responses) to define a reference set of 735 epitopes, of which 484 are recognized by T cells, 344 by B cells, and 93 by both cell types.

Biological Significance and Utility of Reference Epitope Datasets

The discussion sessions in the workshop focused on the biological significance of the information in the epitope database. One topic of particular interest was the potential variation in structure, sequence, and expression of genes encoding epitope-bearing antigens.

Recent characterization of *M. tuberculosis* strains by analysis of large sequence polymorphisms (insertions and deletions) and single-nucleotide polymorphisms has revealed more diversity between strains than previously believed, although strains can be grouped by phylogeography[13–16]. This recently-recognized diversity raises concerns that distinct strains of *M. tuberculosis* may differ in epitope sequences that epitope-based diagnostic assays or subunit vaccines may not take into account. David Alland (New Jersey Medical School, UMDNJ) presented data on sequence polymorphisms in drug target genes that cannot be accounted for by selection for drug resistance, indicating that selection pressures for genetic

variation in *M. tuberculosis* are still not well understood. While an earlier publication revealed limited sequence diversity in 24 antigen-coding genes in 16 strains of *M. tuberculosis* from geographically-distant sources[17], the availability of information on epitopes in the IEDB will facilitate extension of that study to a greater number of antigenic proteins. In turn, there is a need for characterization of epitope sequence variation in diverse strains, in order to better understand mycobacterial evolution, and to apply this information to the design and interpretation of new diagnostic assays and vaccine candidates. Data on these variations (or the lack of them) should be included as data elements in the IEDB.

Michael Brenner (Harvard Medical School) presented data on the structures of the CD1-restricted non-peptide epitopes identified in *M. tuberculosis*: lipoglycans, lipopeptides, mycolic acids. Five of these are restricted by CD1b, and one each by CD1a and CD1c. An existing hindrance to further characterization of the roles of these epitopes in the immune response is lack of an optimal animal model for their investigation.

David Lewinsohn (Oregon Health & Sciences University) provided an overview of the human $CD8^+$ response to M. tuberculosis. These responses appear to be evenly divided between classically (HLA-Ia), and nonclassically (such as HLA-E) restricted responses. Ex vivo characterization of the classically-restricted responses reveals diverse and distinct patterns of immunodominance. Furthermore, these responses are frequently restricted by HLA-B, and consist of 10- to -11-amino acid peptides.

A second topic discussed was the expression of epitope-containing genes. Maria L. Gennaro (Public Health Research Institute) noted studies revealing that expression of several well-characterized epitope genes (*fbpA*, *fbpB*, and *fbpC*) is downregulated during the chronic phase of *M. tuberculosis* infection in mice[18, 19]. Shreemanta Parida (Max Planck Institute for Infection Biology) discussed efforts to define global gene expression profiles of *M. tuberculosis* in vivo, including in human tissues during distinct states of infection. Joel Ernst (NYU School of Medicine) showed data indicating that downregulation of expression of the *fbpB* gene (encoding antigen 85B) in vivo is accompanied by attenuation of $CD4^+$ T lymphocyte responses to that antigen, using adoptive transfer of cells from mice with a T cell antigen receptor transgene that is specific for an epitope of antigen 85B. Juraj Ivanyi (King's College) described results of a published study that found that distinct epitopes of the same *M. tuberculosis* protein (GroES) are recognized with different frequencies by cells of subjects with active or latent tuberculosis[20]. Hardy Kornfeld (University of Massachusetts) presented evidence for heterologous immunity between mycobacteria and certain viruses that influences host susceptibility and immunopathology upon sequential infection. The underlying mechanisms may differ from those reported for heterologous immunity between unrelated viruses, but the data suggest that T cells specific for particular viral epitopes may cross-react with mycobacterial epitopes (and vice versa) with functionally significant consequences.

Additional discussion focused on the utility of reference sets of epitopes for studies of $CD4^+$ and $CD8^+$ T cell and antibody responses in humans, mice, and other experimental animals. Reference sets of epitopes will facilitate comparison of the results of studies in distinct systems and clinical contexts, and will also facilitate development of additional reagents for studies of immune responses to infection and immunization. Sam Behar (Harvard Medical School) advocated the use of define peptide epitopes to evaluate vaccine efficacy. It is important to understand why vaccines fail, whether because of a vaccine failure (no immune response was generated), or because of immunological failure (the immune response that resulted from vaccination does not mediate protection). This is an important role of defined peptide epitopes, as there are several examples in which varying the vaccination strategy for the same antigen led to recognition of different epitopes in that antigen[21]. Whether an epitope is protective or not is determined at least in part by whether it is presented by *M. tuberculosis* infected cells,

and vaccination often elicits T cells recognizing a broader epitope repertoire than elicited following infection[22]. Data was presented showing that a CFP10 DNA vaccine elicits CFP10-specific $CD8^+$ T cells in C3H mice that recognize the same epitope ($CFP10_{32–39}$) that is recognized following *M. tuberculosis* infection. Furthermore, this response protected mice from *M. tuberculosis*, demonstrating that immunological success was linked to protective efficacy. A panel of epitopes recognized by both $CD4^+$ and $CD8^+$ T cells in different mouse strains (C57BL/6, BALB/c, and C3H (e.g., H-2^b,-2^d, -2^k), was presented which can be used for measuring the T cell response in different mouse strains during various experimental situations (vaccination, genetic studies, and differential susceptibility).

Taken together, these findings indicate that the establishment of reference sets of epitopes, and design and development of new vaccines and diagnostic assays, need to take into account the expression, as well as the sequences, of antigenic epitope-coding genes, as it is possible that immune responses to epitopes expressed only early in infection provide little protection during the chronic phase of infection. They also suggest that additional investigation of the functions of immune responses to non-peptide mycobacterial epitopes should be strongly encouraged.

Knowledge Gaps

The participants at the workshop discussed and identified a wide range of existing gaps in knowledge regarding epitopes and immune responses. Several of the topics that received the longest discussion are noted below.

The species and strain specificity of most of the epitopes in the IEDB for mycobacteria is unknown. Epitopes of greatest value for diagnostic tests should be specific for the species of interest, e.g., *M. tuberculosis* or *M. leprae*. In the case of vaccine epitopes, species specificity may be a high priority. For example, epitopes shared by *M. tuberculosis* and *M. leprae* may be useful in a vaccine intended to provide protection against both diseases. In contrast, epitopes shared by *M. tuberculosis* and environmental or opportunistic mycobacteria (or other bacteria) should likely be avoided, especially in a live attenuated vaccine, to minimize interference with vaccine responses.

When possible, information on the strain(s) of mycobacteria used in epitope identification should be provided. When it is not possible to specify details of the strain with which a human subject is infected (such as in those with latent tuberculosis infection), peer-reviewed publications and the IEDB should include information on the likely geographic source of exposure to tuberculosis (the country or region of birth), to allow future inference of strain characteristics, based on knowledge of strains prevalent in the area of exposure. It is also essential in reports of studies of epitope identification using cells from healthy individuals to include information on whether the donors were immunized with BCG, and if so, when and in what country. The IEDB currently provides database fields that allow this information to be recorded where available. However, this level of information is infrequently provided.

The MHC haplotypes that are common in areas with high tuberculosis prevalence should be considered in selecting peptide epitopes for inclusion in diagnostic tests and vaccines. Inclusion of additional information on HLA haplotype data in subjects whose cells are used to identify mycobacterial epitopes should be specified in all published manuscripts describing new epitopes, so that the data can be reflected in the IEDB.

The current knowledge supporting the hypothesis that immune responses and epitope recognition vary with disease state is incomplete. More well-controlled prospective studies are needed that include clear descriptions of the disease state of the donors at the time that cells used for epitope recognition studies were isolated, and information on the existence and nature of identifiable coinfections or comorbidities must be included.

A systematic analysis needs to be undertaken to determine the priority of further investigations of non-peptide epitopes, especially those restricted by nonpolymorphic CD1 molecules. Since complex lipids and lipoglycans represent a large fraction of the total mass of pathogenic mycobacteria, and since these are presented by nonpolymorphic CD1 molecules, their epitopes and their potential roles in protective immunity need to be understood in greater depth.

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