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Review Article

T-cell epitope mapping for the design of powerful vaccines

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ABSTRACT

Epitope mapping has emerged as a powerful tool to design vaccines. It proved itself a number of times to reveal the building blocks of potent immuno-prophylactics. T-cell epitope mapping aims to identify the shortest amino acid sequence of an epitope of a specific antigen that is recognized by CD4+ and/or CD8+ T-cells. T-cell epitopes have the potential to stimulate both long lasting and exclusive cytotoxic immune response. Therefore, they are crucial for vaccine development against emerging intracellular pathogens such as *Plasmodium malaria*, *Mycobacterium* spp., genital *Chlamydia,* HIV, HCV as well as cancer cells. This review documents and discusses the different methods used in T-cell epitope mapping, and the role of each method to identify the potent epitopes in viruses, bacteria, parasites, as well as human diseases. The comment of the article guides the researchers to identify the most suitable mapping techniques for their antigens.

Contents

1. Introduction

When a foreign immunogen is exposed to the immune system, it is first encountered by the antigen presenting cells (APCs) that usually initiate two major immune responses. The B-cells' lymphocytes engulf the foreign body and differentiated into plasma cells, that secrete immunoglobulin antibodies. For this reason this activation is referred as B-cell humoral response. Antibodies recognize foreign molecules called antigens or immunogens and bind to a specific site on the antigen surface called the epitope or the antigenic determinant [1,2]. Unlike B-cells, T-cells do not directly recognize free floating antigens. However, antigens should be first

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up-taken by an APC such as B-cells or dendritic cells or macrophages, and presented to the T-cells by the major histocompatibility complex (MHC). Then the T-cells receptor (TCR) recognizes the fragments exposed by the APCs and initiates the T-cell response [3,4].

The T-cell-mediated immune response depends on four different types of T-cells lymphocytes. The cytotoxic T- lymphocytes (CTL), or killer T-cells play a unique role to destroy cells displaying foreign motifs on their surfaces. Whereas helper T-cells (HTC), contribute to both the humoral and the cellular immune responses by stimulating the proliferation and differentiation of B-cells and killer T-cells. In addition, regulatory T-cells function in maintaining tolerance toward self-antigens, playing an important role in autoimmune diseases. While, memory T-cells are responsible for maintaining a relatively long lasting immunological memory towards the same antigen [1,2,5].

The CTL expresses a cluster of differentiation (CD) receptor glycoprotein termed CD8+ on its surface, which recognizes the MHC class I-peptide complex displayed by the APCs. Association of MHC class I-peptide complex with the cytotoxic TCR, and other accessory proteins, aids in activating a series of reactions that result in apoptosis of the infected cell by forcing the cell to suicide [4,6,7]. On the other hand, the HTC expresses a specific glycoprotein on its surface termed CD4+ that has the ability to recognize class II MHC-peptide complex. Association of class II MHC-peptide complex with the helper TCR gives signals that a pathogen is invading the cell. The initiation, differentiation and regulation of those responses trigger the secretion of small cell-signal molecules referred to as cytokines [3,8]. Cytokines (CK) are functionally classified into a T-cell helper 1 (Th1) group that enhances the cellular immune response such as interferon-γ (IFN-γ) and interleukins (IL-12 and IL-18), and a Th2 group that enhances the humoral immune response such IL-2, IL-4 and IL-10 [9]. Those can be measured to monitor the cellular immune response [10].

The design of vaccines and diagnostics is a recent art that attracted the attention of scientists worldwide [11]. Epitope mapping is a new branch of immunology which focuses on the selection of the most potent epitopes that could serve as potential targets for the production of epitope-based immune-preparations. Several occasions highlighted the advantages of following the route to design vaccines and diagnostics [12–15]. T-cell epitope mapping is a term that refers to the process of identifying the shortest amino acid sequence of the epitope of a specific antigen that is recognized by CD4+ and/or CD8+ T-cell receptors, and at the same time has the potential to stimulate a long lasting and a cytotoxic immune response [16,17]. Therefore, T-cell epitope mapping is critical for constructing epitope-based vaccines as well as potent therapeutics for intracellular pathogens and cancer cells [9,17–19]. Unlike the majority of B-cell epitopes, T-cell epitopes are linear protein molecules that consist of 12 to 20 amino acids. This fact facilitates their cloning and their synthesis as peptide vaccines [16]. Some methods may be used for both T- and B-cell epitope mapping such as pepscan, *in silico*, ELISA and ELISPOT that corresponds to the microarray in B-cell epitoping [14]. Simultaneously, new techniques such as mRNA [20] and random phage display library [21] are under investigation.

The selection of a target epitope that has the ability to induce a protective B-cell dependent immune response is crucial for vaccine development [5,7,18], however this will not always lead to the establishment of long lasting protective memory inside the host [22]. Moreover, in the case of intracellular pathogens and tumors the cellular arm of the immune response managed by T-cell activity, is highly involved in the process of fighting the foreign bodies as well as inducing a cytotoxic activity [4,23]. The generation of memory T-cells enables the rapid recovery of the host and the prevention of re-infection by the same pathogen [23]. In addition, it was shown that the integration of the action of cell-mediated immunity effectors such as IFN-γ, Natural Killer (NK) cells, as well as Th1 helper T-cells are required in the control of the pathogenesis of intracellular microbes such as *Rickettsia* [24], *Salmonella* [25], *Listeria* [26], Mycobacteria [27], and *Chlamydia* [28,29], as well as allergens [16]. On the other hand, CD8+ T-cells are highly involved in providing immune protection against respiratory viruses [30], *Vaccinia* virus [31], *Plasmodium* spp. [32–35], *Toxoplasma gondii* [36], and intracellular bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* [7,37,38]. Moreover it was noted that, B-cell-deficient mice were able to sustain complete recovery from genital tract infection with *Chlamydia trachomatis* [39], which ensures the essential role of T-cells in the recovery from some diseases.

Although the development of a T-cell protection is the corner-stone for constructing potent vaccines [5,10,18], more recent studies showed that cellular response is not sufficient for the complete irradiation of intracellular pathogens such as *Chlamydia* and *M. tuberculosis*. A comprehensive immunological study showed that long lasting persistence and increased susceptibility of *Chlamydia* and Mycobacteria infections appeared in the B-cell deficient models [38,39]. Therefore, it was found that antibodies, as well, play an indirect role in T-cell activation, thus resulting in early and effective clearance of the pathogen in the process of secondary infection. In addition in small pox and *Ebola* virus [40] it was found that although the elucidation of potent cellular immune response is required for the rapid recovery of infected individuals, the activation of the humoral response is essential for protection [41,42]. Therefore, the novel approach in the design of vaccines is to implement strategies capable of activating both arms of the immune system, the cellular as well as the humoral immune responses [13]. The different techniques followed to map the T-cell epitope are described hereafter, in order to integrate them to the mapping techniques of the B-cells [14].

2. Methods of T-cell epitope mapping

Several techniques depending on *in silico* and binding methods developed to point the epitopes that activate the cellular response. The following paragraphs discuss those methods and document the majority of the practical trials that involved their use. The T-cell epitope mapping techniques were the corner-stone to reveal the potent antigenic determinants of many intracellular bacteria, viruses, cancer, autoimmune, allergen and parasites' antigens.

2.1. MHC Multimers (MM)

This method depends on synthetic MHC molecules that bind to the tested peptide and propose it to the TCRs. Hence it isolates and identifies the bound T-lymphocytes, in case the tested peptide is an appropriate epitope [43]. The most widely spread multimers, are MHC tetramers composed of four MHC molecules covalently attached to biotin [44]. The first approach that used this technique allowed the peptide antigen to attach to the tetramer-complex. Further coupling was detected by a suitable fluorescent tag that has a high affinity to biotin such as streptavidin, then the emitted fluorescence was measured [45]. Although this method is simple and recoverable as the cells can be reused for flow cytometry studies, it has some drawbacks. Since, the antigens had to be known in advance using some other methods of epitope mapping, and the prior knowledge of MHC-peptide interactions is needed in order to design suitable tetramer components [46]. This method developed to apply a reversible binding between the peptide and the MM. In this later approach, a peptide that has a UV cleavage site