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Review Article

B-cell epitope mapping for the design of vaccines and effective diagnostics

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ABSTRACT

The increasing resistance of many microbial strains to antibiotics, delayed laboratory results, and side effects of many chemotherapeutics has raised the need to search for sensitive diagnostics and new prophylactic strategies especially prevention by vaccination. Understanding the epitope/antibody interaction is the key to constructing potent vaccines and effective diagnostics. B-cell epitope mapping is a promising approach to identifying the main antigenic determinants of microorganisms, in special concern the discontinuous conformational ones. Epitope-based vaccines have remarkable privilege over the conventional ones since they are specific, able to avoid undesirable immune responses, generate long lasting immunity, and are reasonably cheaper. This up-to-date review discusses and compares the different physical, computational, and molecular methods that have been used in epitope mapping. The role of each method in the identification of potent epitopes in viruses, bacteria, fungi, parasites, as well as human diseases are tagged and documented. Simultaneously, frequent combinatorial methods are highlighted. The article aims to assist researchers to design the most suitable protocol for mapping their B-cell epitopes.

Contents

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1. Introduction

Immunology has a great impact on improving global health through the synthesis of rapid diagnostics. Simultaneously, it paved the road towards the protection, as well as the complete eradication, of many pathogens via the art of vaccination [1,2]. Antibodies (Abs), also known as immunoglobulins (Igs) are essential components of the immune system of all vertebrates. They are able to identify and neutralize foreign immuno-stimulating objects such as viruses, bacteria, parasites, fungi, cancer cells, and some toxins by binding to specific parts on their surface, that are usually called antigens (Ag) [3]. In fact, the paratope, a specific part of an antibody, binds to a particular region on the antigen that is called the epitope or the antigenic determinant [3,4]. Unlike the T-cell epitopes, the majority of the functional B-cell epitopes are discontinuous non-linear epitopes having 3D-conformational structures [5]. The studies of paratope–epitope interaction are considered recent [6]. Their study offers benefits to the fields of research in immune response, vaccines and diagnostics design, passive immunization, allergens, and auto-immunity [7,8].

The production of diagnostics and immunotherapeutics first depended on the use of the whole antigen upon *trial and error* methods or virulence studies guidance [2,9]. However, the degree of success of any epitope depends on its ability to induce the most specific and detectable rapid immune response in the case of diagnostics. While it lies on its capability to confer a neutralizing safe response for vaccines (B-cell dependent response) [10]. Moreover, its ability to stimulate cytotoxic or long-lasting potent immune response (T-cell dependent response) for vaccine production as well [11]. Therefore, epitope mapping has developed in order to focus on the selection of the most potent epitopes that could serve as potential targets for the production of epitope-based diagnostics and vaccines [1,4,12]. A special concern is dedicated for DNA vaccines that are built upon short peptide chains [13]. It was noticed that the presentation of non-protective epitopes deviates the immune system potency [1,14,15], and decreases the antigen–antibody affinity [16]. Therefore, epitope-based vaccines aim to provide protective focused immunity without mimicking the host self-antigens, which render them safe regarding autoimmune disease induction [1,14,15]. Vaccines' mapping aid in reducing the cost, complexity, and time of synthesis [1].

Although B-cell epitope mapping is the corner stone-step in the production of diagnostics, it is only the first step to design potent vaccines [1,10,12,17]. Epitope mapping will not lead to the straight forward identification of highly protective monoclonal antibodies (mAbs), as some antibodies that showed neutralizing activity *in vitro* were not able to do so *in vivo*. Moreover, they can give rise to *in vivo* antibody dependent enhancement (ADE), a phenomenon that increases the infectivity of host cells to viruses in the presence of some antiviral antibodies [18]. In addition to this, several epitope mapping techniques cannot differentiate between conformational and linear epitopes. Thus, careful considerations have to be taken when analyzing the results acquired by different methods used in mapping [1,4,19]. Furthermore, results obtained could be enhanced and confirmed by combining two or more techniques of epitope mapping [20,21]. These results may be consolidated by the T-cell ones, especially for the vaccine development against cancer [22,23] or intracellular pathogens [24–26].

2. The different methods for B-cell epitopes' mapping

The following paragraph documents the different physical, computational and binding methods used in B-cell epitope mapping for vaccines and diagnostics production.

2.1. Crystallography-based methods

Co-crystallization of antigen–antibody complex is one of the first techniques that were used in epitope mapping. In this technique, the highly purified antigens are obtained and allowed to co-crystallize with their corresponding antibodies. Then, the atomic structure of the complex is solved using X-ray diffraction analysis. The structure of the epitope is obtained by solving the three dimensional coordinates that represent the electron densities of the amino acids of the antigen–antibody complex [1,10,27]. The amino acids that are within a distance of 4 Å of each other are considered to be counteracting [6]. Unlike several techniques of B-cell epitope mapping, the co-crystallization method is able to detect continuous linear epitopes as well [4,10]. However, the technique is obviously complex and expensive, as it requires large amounts of highly purified protein-mAbs (monoclonal antibodies) complexes and the structure of the mAbs should be known [1,28]. Obviously the antigen–antibody complex must be crystallizable [29]. Thus some antigens are still mapped by this technique to reveal the B-cell epitopes of malaria [30] and bilharzias [31] parasites.

Thoughts to apply electron-microscopy (EM) to reveal the antigen–antibody interaction were not practical, since the complex-molecule was subjected to degeneration and dryness [32]. Since the beginnings of 2013 the progress in cryo-EM analysis technique re-introduced the use of EM to study the freezed antigen–antibody complex in a non-crystalline amorphous thin layer, especially when coupled with X-ray crystallography [33]. The new technique requires less amount of complex, do not necessitate high purity of the complex's components, and the ability of the complex to crystalize is not a must [32]. This technique was recently used to map the B-cell epitopes of HIV-1 [34] and HPV [35], or in combination with X-ray crystallography to map poliovirus type-1 and 2 [36,37] and to study the structure of the rabbit hemorrhagic virus [38] for vaccines' production.

2.2. Mass spectrum-based methods

The application of mass spectrum (MS) in epitope mapping had positively influenced the identification and characterization of discontinuous epitopes [39]. In general, there are two main methods to use MS in epitope mapping. The limited proteolysis method, in which different proteases are applied to the antigen of interest. The fragments released from the different cleavage-sites in the presence and absence of the antibody are detected by MS to reveal the bound fragments to the antibody [40]. While in the epitope excision method extensive proteolytic digestion is applied to the antigen incubated with antibody coated beads. The beads are then washed for several times to eliminate the non-epitope fragments leaving the epitope fragments bound to the antibody. The epitopes are then liberated by acid washing for further identification by LC-MS or MALDI/MS [41,42]. One of the major limitations of this method is the resolution which is, the ability to accurately determine a peptide fragment. The reason is that the limited proteolysis and epitope excision approaches require proteolytic digestion with trypsin. Therefore, epitope mapping is confined only to cleavage sites which results in the identification of long peptides (typically 30–60 residues) that poorly define the epitopes. In addition, the method is more concerned by the location of the reactive residues on the antigen of interest rather than the spacing between them [39], that shares in the conformational structure of the epitope.

The antigen–antibody complex placed in deuterated solvent, will exchange the deuterium atoms with the free non-bound sites. After digestion with pepsin the level of deuteration may be estimated by MS [43]. Hydrogen–deuterium exchange method coupled to MS (HD-MS) technique was used to overcome the defects