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Deciphering epitope specificities within polyserum using affinity selection of random peptides and a novel algorithm based on pattern recognition theory

Dimitri A. Denisov*, Galina F. Denisova, Alina Lelic, Mark B. Loeb, Jonathan L. Bramson

Department of Pathology and Molecular Medicine, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5

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ABSTRACT

While numerous strategies have been developed to map epitope specificities for monoclonal antibodies, few have been designed for elucidating epitope specificity within complex polysera. We have developed a novel algorithm based on pattern recognition theory that can be used to characterize the breadth of epitope specificities within a polyserum based on affinity selection of random peptides. To attribute these random peptides to a specific epitope, the sequences of the affinity-selected peptides were matched against a database of random peptides selected using well-described monoclonal antibodies. To test this novel algorithm, we employed polyserum from patients infected with West Nile virus and isolated 109 unique sequences which were recognized selectively by serum from West Nile virus-infected patients but not uninfected patients. Through application of our algorithm, it was possible to match 20% of the polyserum-selected peptides to the database of peptides isolated by affinity selection using monoclonal antibodies against the virus envelope protein. Statistical analysis demonstrated that the peptides selected with the polyserum could not be attributed to the peptide database by chance. This novel algorithm provides the basis for further development of methods to characterize the breadth of epitope recognition within a complex pool of antibodies.

1. Introduction

Antibodies play a central role in immune memory and long-term protective responses. Recent studies have revealed that antibody responses persist for decades following vaccination (Amanna et al., 2007). While increased serum antibodies for specific pathogens are recognized as a primary read-out for vaccination, it is important to recognize that not all antibodies can prevent infection. As an example, a broad range of monoclonal antibodies have been isolated against the West Nile virus envelope protein (E) which recognize multiple distinct epitopes within the protein. However, only the binding of specific epitopes will result in virus neutralization and protective immunity *in vivo* (Oliphant et al., 2007). Therefore, to properly characterize a protective humoral response, it is necessary to characterize the epitope specificity of the antibodies in the patient.

Given the broad range of potential epitopes within a protein, characterizing the breadth of epitopes recognized by a particular polyclonal response is quite challenging. A typical strategy for monitoring epitope-specific reactivity would be the production of recombinant proteins or synthetic peptides which only carry a single epitope and subsequent analysis of antibodies specific for these epitopes using standard methods such as ELISA. Ideally, however, it is desirable to take an unbiased approach to this analysis where the entire repertoire of the polyserum is examined. To this end, we have explored the utility of affinity selection of random peptides as a means of characterizing the breadth of epitopes recognized in polyserum. We have previously demonstrated that affinity selection of random peptides can be used to characterize the target epitopes of monoclonal antibodies (Denisova et al., 1995, 1996, 2003, 2000, 2008; Enshell-Seijffers et al., 2003; Gershoni et al., 1997; Paley et al., 2007; Stern et al., 1997). Manipulation and identification of random peptides is facilitated by the use of phage-display libraries (Smith and Petrenko, 1997) where random 10-mer peptides flanked by cysteine residues are presented as circularized peptides on the phage surface (Scott and Smith, 1990). The random peptides presented by phage display can represent peptidomimetics of conformational and discontinuous antibody epitopes (Felici et al., 1993; Luzzago et al., 1993) referred to commonly as “mimotopes”. Isolation of specific mimotopes is accomplished through affinity selection where antibodies of interest are first immobilized onto a solid matrix and subsequently reacted with the phage library expressing the mimotopes. Iterative washing and binding steps allows for enrichment of phage carrying mimotopes which are specific for the antibodies.

* Corresponding author at: Department of Pathology and Molecular Medicine, McMaster University, Room MDCL-5071, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. Tel.: +1 905 525 9140; fax: +1 905 522 6750.
E-mail address: bellalel@hotmail.com (D.A. Denisov).

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The mimotope sequences are then analyzed and assigned a location on the target protein using algorithms that we have designed based on specific correlation analysis (Enshell-Seijffers et al., 2003). We have recently employed this method for the identification of four epitopes in the West Nile virus E protein using monoclonal antibodies (Denisova et al., 2008).

Unfortunately, mixtures of antibodies with discrete specificities create a significant hurdle for our published algorithm due to the statistical principles that were employed for defining specific epitopes (Enshell-Seijffers et al., 2003). Therefore, we have developed a novel method for attributing epitope specificity to affinity-selected mimotopes based on pattern recognition theory (Bongard, 1970) (Denisov, 1975; Denisov et al., 1973). To attribute mimotopes selected by a mixture of antibodies to a single antibody, we identify "signs" that are uniquely present within the amino acid sequences of the specific antibody epitope. For the algorithm described in this manuscript, we have employ amino acid pairs chosen from different positions within an epitope sequence as "signs" and identify unique signs which are specific to individual epitopes.

In principal, these signs could be found by theoretical epitope prediction software (Greenbaum et al., 2007). While this is our ultimate goal, currently those methods for epitope prediction remain imperfect. Therefore, to test our novel algorithm, we have employed a model system where signs could be identified within a collection of defined, affinity-selected mimotopes. To this end, we have chosen to investigate the epitope-specificity of antibodies against the West Nile virus E protein in polyserum obtained from infected patients by matching the signs to a recently catalogued database of mimotopes that bind to monoclonal antibodies specific for E (Denisova et al., 2008). In this manuscript, we demonstrate our novel algorithm can successfully assign polyserum-selected mimotopes to defined epitopes on the E protein.

2. Materials and methods

2.1. Preparation of polyclonal serum

Sera from 20 West Nile virus (WNV)-infected individuals were pooled in order to identify the most prominent polyclonal antibody epitopes. The relative anti-WNV antibody concentration in each sample was quantified by Western blot against WNV-infected Vero cell lysate. Lysates of VERO cells infected with WNV (gift of M. Diamond) were electrophoresed through 10% SDS-PAGE, transferred onto nitrocellulose membrane, blocked with 5% non-fat milk solution in Tris-buffered saline (pH 7.4). The blot was subsequently hybridized with varying dilutions of serum from individual patients and specific antibodies were detected following incubation with peroxidase-conjugated goat anti-human IgG (Jackson Immunoresearch Laboratories Inc., PA, USA) and development using ECL (Amersham Biosciences, NJ, USA). The intensity of the protein band corresponding to E was determined using densitometry and this information was used to define the titer of E-specific antibodies in each polyserum. Approximately equal amount of E-specific antibodies from each sample were pooled and used for affinity selection. Since human serum contains a fraction of phage-specific antibodies, the pooled serum was adsorbed to immobilized phage f88-4 to remove phage-specific antibodies (Scott and Smith, 1990). This process was repeated three times and the non-adsorbed serum, which was now free of anti-phage antibodies, was used for subsequent affinity selection.

2.2. Affinity selection of mimotopes

A phage display library presenting random, circularized 10-mer peptides with the structure $XC(X)_{10}CX$ inserted into N-terminus of pVIII major coat protein of filamentous bacteriophage, where X represents any amino acid (kindly provided by Dr. J. Scott, Simon Fraser University) (Bonnycastle et al., 1996) was employed for this step. Screening of phage library was performed according to the method described by Smith (2006). Since many of the antibodies present in the pooled serum from our West Nile virus patients will be specific for common environmental pathogens and vaccines, the phage library was first depleted of phage particles reactive to normal human serum (pooled from five non-infected individuals) by adsorbing the phage library to normal human antibodies bound to Protein G-sepharose. The bound phages were removed by centrifugation of the immune complexes and the process was repeated three times. This procedure ensured that we eliminate phage particles reactive with normal serum antibodies.

The depleted phage library was reacted with the depleted polyserum described in the previous section. Bound phages were selected and subjected to iterative cycles of adsorption to the depleted polyserum. Following the third round of selection, we identified phages that bound only to serum from West Nile virus infected patients and not to serum from uninfected patients. An example of such phages is presented in Fig. 2B. This process yielded 109 unique phage sequences that served as the basis of our study.

2.3. Algorithm

Let us consider as an example two groups of peptides: Groups A and B.

Group A consists of mimotopes isolated by affinity selection using a specific monoclonal antibody. The premise for our algorithm is that certain similarity should exist between all the peptides in Group A (despite the absence of clear linear homology) since they interact with the same antibody binding site. However this type of homology cannot be elucidated by simple alignment of the peptide sequences (Valencia, 2003) in contrast to linear homology (homology type 1). We will refer to our non-linear homology as "type 2". We develop here a simple method of type 2 peptide homology estimation based on comparison of the amino acid pair compositions within the Group A mimotopes. The definition of amino acid pairs includes not only neighboring amino acids, but also pairs of amino acids which are remote in the peptide sequence since it is possible that folding of the mimotope may bring such residues into spatial proximity of each other. Common characteristics within the Group A mimotopes should be reflected in their pair compositions. The greater the commonality among the Group A mimotopes, the greater the likelihood that these pairs could serve as common features (signs) of these peptides.

Group B consists of mimotope peptides selected by affinity-purification using polyserum. Unlike the Group A mimotopes, the Group B mimotopes were selected by multiple monoclonal antibodies of differing specificity. In that case, we cannot simply look for commonalities within the Group B mimotopes to assign these mimotopes to a specific epitope.

The problem of identifying commonalities within the complex collection of Group B mimotopes may be addressed using the pattern recognition method. The recognition methods are developed now for many applications (Lorenz et al., 2008; Raykar et al., 2008; Schlecht et al., 2008; Shotton et al., 2008; Ullman, 2007). General approach to recognition problem was described by Bongard (1970). Let us consider the main features of a recognition method used in this study (Fig. 1).

- Step 1. *Learning*

We analyze the Group A mimotope peptides isolated by affinity selection using specific monoclonal antibodies ("material for learn-