Vaccine 27 (2009) 5740–5747

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Immunoinformatic comparison of T-cell epitopes contained in novel swine-origin influenza A (H1N1) virus with epitopes in 2008–2009 conventional influenza vaccine

Anne S. De Groot[a,b,c,*], Matt Ardito[a], Elizabeth M. McClaine[a], Leonard Moise[a,b], William D. Martin[a]

[a] EpiVax, 146 Clifford Street, Providence, RI 02903, USA
[b] Institute for Immunology and Informatics, University of Rhode Island, 80 Washington Street, Providence, RI 02903, USA
[c] Alpert Medical School, Brown University, Providence, RI 02903, USA

ARTICLE INFO

Article history:
Received 6 June 2009
Received in revised form 6 July 2009
Accepted 10 July 2009
Available online 4 August 2009

Keywords:
H1N1
Influenza
T-cell epitope
Immunoinformatics
Algorithm
Cell-mediated

ABSTRACT

In March 2009 a novel swine-origin influenza A (H1N1) virus (S-OIV) emerged in Mexico and the Western United States. Vaccination with conventional influenza vaccine (CIV) does not result in cross-reactive antibodies, however, the disproportionate number of cases (37%) occurring among persons younger than 50 years old suggested that adaptive immune memory might be responsible for the relative lack of virulence in older, healthy adults. Using EpiMatrix, a T-cell epitope prediction and comparison tool, we compared the sequences of the three hemagglutinin (HA) and neuraminidase (NA) proteins contained in 2008–2009 CIV to their counterparts in A/California/04/2009 (H1N1) looking for cross-conserved T-cell epitope sequences. We found greater than 50% conservation of T helper and CTL epitopes between novel S-OIV and CIV HA for selected HLA. Conservation was lower among NA epitopes. Sixteen promiscuous helper T-cell epitopes are contained in the S-OIV H1N1 HA sequence, of which nine (56%) were 100% conserved in the 2008–2009 influenza vaccine strain; 81% were either identical or had one conservative amino acid substitution. Fifty percent of predicted CTL epitopes found in S-OIV H1N1 HA were also found in CIV HA sequences. Based on historical performance, we expect these epitope predictions to be 93–99% accurate. This in silico analysis supports the proposition that T-cell response to cross-reactive T-cell epitopes, due to vaccination or exposure, may have the capacity to attenuate the course of S-OIV H1N1 induced disease—in the absence of cross-reactive antibody response. The value of the CIV or live-attenuated influenza vaccine containing the 2008–2009 vaccine strains, as defense against H1N1, could be further tested by evaluating human immune responses to the conserved T-cell epitopes using PBMC from individuals infected with H1N1 and from CIV vaccinees.

1. Introduction

The emergence of swine-origin influenza A (H1N1) virus (S-OIV) [1] as a global epidemic [2] is due in part to differences between the hemagglutinin (HA) sequence of the S-OIV and conventional influenza vaccine (CIV) strains and the susceptibility of most human populations to this new strain of influenza. The differences between the hemagglutinin (HA) sequence of the S-OIV and the CIV vaccine strains became clear when individuals vaccinated against H1N1 viral strains, by CIV, failed to produce cross-reactive antibodies to the new H1N1 influenza [3]. This lack of cross-reactivity suggested that the existing vaccine might not provide effective cross-protection, setting up a "perfect storm" in terms of potential for widespread disease and significant economic impact. Compounding this concern was the fact that that novel S-OIV H1N1 is resistant to amantadine and rimantadine [4]. Resistance to the two remaining licensed antivirals, oseltamivir and zanamivir, following potential spread of the H1N1 epidemic in the southern hemisphere, has been an additional concern raised by the WHO and the CDC [5].

The 2008–2009 seasonal CIV was composed from the HA and NA proteins of three types of viruses: A/Brisbane/59/2007 H1N1, A/Brisbane/10/2007 H3N2 and B/Florida/4/2006. HA and NA from these three viruses were also used to develop the southern hemisphere influenza vaccine for 2008–2009. More than 130 million individuals in the United States have had access to the current vaccine and more than 1 billion doses were manufactured for distribution worldwide, with an estimated 500 million doses used [6].

The 2008–2009 seasonal CIV contained an H1N1 virus (A/Brisbane/59/2007). Novel S-OIV A/California/04/09 shares only

* Corresponding author at: EpiVax, 146 Clifford Street, Providence RI 02903, USA. Tel.: +1 401 272 2123; fax: +1 401 272 7562.
E-mail address: AnnieD@EpiVax.com (A.S. De Groot).

0264-410X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2009.07.040

72–73% amino acid identity [7]. In contrast, the amino acid sequence identity in the HA portion among seasonal vaccine strains (2007–2008 and 2008–2009) is 97% and 98%, respectively. The amino acid sequence divergence between HA-H1 of A/California/04/09 and HA-H1 of A/Brisbane/59/2007 probably contributes to the lack of antibody cross-reactivity detected among individuals immunized with 2008–2009 CIV [3]. While overall HA and NA sequence changes are of great significance in terms of antibody response, immunological differences are also expressed on the level of T-cell epitopes, short peptide sequences recognized by T cells in the context of HLA molecules.

T-cell responses to conserved epitopes may be particularly important when new strains of influenza emerge. In mice and in humans, memory T cells to conserved epitopes have been shown to confer protection to heterotypic infection [8,9]. The activation of Th cells is also critically important to the magnitude, quality and kinetics of antibody response [10]. In the absence of functional (memory) $CD4^+$ T cells, studies in mice have shown that the rate of viral clearance upon secondary infection slows considerably, beyond the degree seen in the primary response [11–13]. Also in mice, cross-reactive memory T-helper cells have been shown to contribute to cross-strain antibody responses [14]. In human populations, cross-reactive T-cell responses have been demonstrated between circulating strains of influenza and epidemic strains (such as H5N1) in the absence of cross-reactive antibodies [15]. Both cross-reactive CTL and T helper cells have been identified by a number of investigators [16,17].

A number of mechanisms could contribute to the relative lack of severe disease among older adults in areas where pandemic S-OIV is circulating. One potential explanation for protection against severe disease in the absence of cross-reactive antibodies might be the presence of cross-reactive T-cell response. Therefore, we decided to determine whether cross-conserved T-cell epitopes might be present in the novel A/California/04/09 sequence. So as to get the most immediate estimate of the potential for the existing CIV to protect against the emerging S-OIV, we examined the protein sequences of S-OIV and CIV 2008–2009 in silico, using well validated immunoinformatics tools. Herein, we describe a set of cross-reactive T-cell epitopes that are relatively well conserved between the novel S-OIV and the existing seasonal influenza vaccine strains. We provide a list of potential cross-reactive T-cell epitopes (and epitopes unique to the CIV and epidemic strains) that may be synthesized and used to confirm cross-reactivity between CIV and the epidemic strain in vitro, using peripheral white blood cells (PBMC) from exposed and vaccinated donors.

The overall average predictive efficacy of a number of epitope mapping algorithms, considering a range of viral and bacterial pathogens, has been shown to be as high as 93% for Class II epitopes and higher for Class I epitopes [18,19]. As shown here, many of the conserved influenza epitopes defined by our tools have been previously published. Thus, computationally identified cross-reactive epitopes are a good starting point for further immunological and immunoprophylactic studies. We expect that the epitopes described will be confirmed in standard in vitro assays and that they might also be useful for the development of a novel epitope-based "cross-strain" influenza vaccine. Production of such a vaccine may be more rapid and efficient than egg- or cell culture-based vaccines in the context of an emerging epidemic.

2. Methods

2.1. Epitope mapping

EpiMatrix, a T-cell epitope mapping algorithm developed by the principal scientists at EpiVax, screens protein sequences for 9–10 amino acid long peptide segments predicted to bind to one or more MHC alleles [20,21]. EpiMatrix uses the pocket profile method for epitope prediction, which was first described by Sturniolo and Hammer in 1999 [22]. For reasons of efficiency and simplicity, predictions are limited to the eight most common HLA Class II alleles and six "supertype" HLA Class I alleles [23]. EpiMatrix raw scores are normalized with respect to a score distribution derived from a very large set of randomly generated peptide sequences. Any peptide scoring above 1.64 on the EpiMatrix "Z" scale (approximately the top 5% of any given peptide set) has a significant chance of binding to the MHC molecule for which it was predicted. Peptides scoring above 2.32 on the scale (the top 1%) are extremely likely to bind; the scores of most well known T-cell epitopes fall within this range of scores [24–26]. EpiMatrix has been successfully applied to the analysis of previously published epitopes [27], and in the prospective selection of epitopes from HIV [28], Mycobacterium tuberculosis [29], Tularemia [30] and vaccinia virus [31]. An ancillary algorithm, ClustiMer, identifies "clustered" or promiscuous epitopes [29,30]. Conservatrix compares similar sequences (between strains) [32] and BlastiMer identifies homologies between the putative epitopes identified by EpiMatrix and any protein sequence on file at GenBank [23].

2.2. Influenza sequences

HA and NA sequences were obtained from the National Center for Biotechnology Information (NCBI). The downloaded sequences were from A/California/04/2009 H1N1 GENBANK: NA ACP41107; HA ACP41105), A/Brisbane/59/2007 H1N1 (GENBANK: NA ACA28847; HA ACA28844), A/Brisbane/10/2007 H3N2 (GENBANK: NA ACO95273; HA ACO95270), and B/Florida/4/2006 (GENBANK: NA ACA33351; HA ACA33493). The novel S-OIV H1N1 sequences analyzed were selected from among those posted to GenBank on April 29th, 2009.

2.3. Conservation

We used the Conservatrix algorithm to parse and compare 9-mers for segments that were highly conserved between the epidemic strain and CIV. In assessing HLA specific conservation we allowed up to two amino acids out of any 9-mer frame to vary, as long as the variant peptide was still predicted to bind to the target HLA allele. This allowance is supported by studies that indicate that there may be even greater flexibility in the binding of T-cell receptors to peptides that bind in the HLA groove than previously recognized [33,34].

2.4. HLA restriction

The HLA alleles selected for this analysis were selected to maximize cross-HLA coverage [32,35]. Both DeLisi and Sette have addressed the issue of HLA coverage for epitope predictions by demonstrating that epitope-based vaccines that contain epitopes restricted by selected "supertype" HLA can provide the broadest possible coverage of the human population [36]. The set of six, HLA Class I alleles used in this analysis on average, cover over 99% of the population when considering Caucasian, North American Black, Japanese, Chinese, and Hispanics. The eight "supertype" Class II used also cover over 99% of the human population. In this analysis we have limited our Class II epitope predictions on "promiscuous epitopes'—epitopes that are recognized in the context of more than one HLA [37–39]. We focuses our analysis on regions of the HA and NA sequences that contained more than four potential Class II HLA binding motifs.