Options for the Control of Influenza VIII

Cape Town, South Africa
5-10 September 2013

Abstracts

For full instructions on how to best view and search this document, please see the READ ME Instructions included on this USB.

www.controlinfluenza.com

isirv
International Society for Influenza and other Respiratory Virus Diseases

LBA-P2-040

A broadly protective monoclonal antibody targeting a universally conserved epitope in all influenza A neuraminidase subtypes

TM Doyle[1,2], AM Hashem[1,2,3], B Jaentschke[1], C Li[4], G Van Domselaar[5], L Larocque[1], NE Forbes[2], J Wang[4], D Smith[1], T Cyr[1], A Farnsworth[1], R He[5], AC Hurt[6], RG Brown[2,7], X Li[1,2,7*]*

[1]Centre for Vaccine Evaluation, Biologics and Genetic Therapies Directorate, HPFB, Health Canada, Ottawa, Ontario, Canada; [2]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada; [3]Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia; [4]National Institutes for Food and Drug Control, Beijing, China; [5]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada; [6]WHO Collaborating Centre for Reference and Research on Influenza, North Melbourne, Victoria, Australia; [7]Emerging Pathogens Research Centre, University of Ottawa, Ottawa, Ontario, Canada

Background: Hemagglutinin (HA) and neuraminidase (NA) are the two major surface glycoproteins of influenza A virus and the main targets of protective immune response. Current seasonal influenza vaccines can induce strain-specific neutralizing anti-HA antibodies (Abs). In contrast to HA, anti-NA Abs can reduce viral yield and spread by inhibiting NA enzymatic activity. While several broadly neutralizing Abs targeting conserved epitopes in diverse HA subtypes have been isolated, NA-specific antibodies could only cross-protect partially against homologous and heterologous strains from the same subtype. Thus, we tried here to identify highly conserved sequences in all influenza NA subtypes and determine their functional roles in viral replication and their potential as antiviral targets. Materials and Methods: Comprehensive bioinformatics analyses of all publicly available full-length NA sequences using multiple alignments and Shannon entropy analyses were conducted to identify conserved sequences in all NA subtypes. Reverse genetics (RG) recombinant viruses were generated containing the 7 genes of influenza A/Puerto Rico/08/34(H1N1) and either the wild-type (wt) NA gene or NA plasmids with single alanine substitutions within the identified conserved regions. Growth kinetics of wt virus and each RG variant was then analyzed in MDCK cells. Thermostability and enzymatic activity were determined using the 2-(4-methylumberlliferyl)-α-D- N-acetylneuraminic acid (MUNANA) assay. A rabbit monoclonal Ab, denoted as HCA-2 mAb, raised against one of the characterized sequences was then examined for its inhibitory effects against replication and enzymatic activity of wt viruses from all 9 NA subtypes as well as the RG variants using in vitro microneutralization and enzyme-linked lectin assay, respectively. Finally, in vivo prophylactic efficacy of HCA-2 mAb was tested in a murine model against lethal doses (5 x LD_{50}) of influenza A/FM/1/47/MA (H1N1) and influenza A/HK/1/68-MA20 (H3N2) viruses. Results: Multiple sequence alignments and Shannon entropy analyses of all NA sequences in the GenBank uncovered a universally conserved 9-mer peptide amongst all influenza A viral NA subtypes located between amino acids 222 and 230 (N2 numbering) and comprised of “ILRTQESEC.” Substitutions within this universal epitope significantly affected viral fitness by decreasing the catalytic activity, substrate binding and thermostability of the NA enzyme. Consistently, protein modeling suggested that these residues could either directly bind to the substrate or contribute to the formation of the active site in the enzyme. Importantly, the HCA-2 mAb, which targets this universal peptide, significantly decreased NA enzymatic activity and showed broad in vitro inhibitory effect against antigenically diverse influenza A strains from all NA subtypes (N1-N9). It also provided heterosubtypic protection in mice challenged with lethal doses of mouse-adapted H1N1 and H3N2 strains (representing group 1 and 2 viruses, respectively). Finally, we found that amino acid residues I222 and E227, located in close proximity to the active site of the enzyme, are indispensable for inhibition by this mAb. Conclusions: Collectively, these findings not only reveal the essential role of this unique highly conserved linear sequence in NA function and viral replication, but also provide the basis for evaluating the potential of this sequence in the development of targeted vaccines or in the design of novel antivirals. Moreover, the HCA-2 mAb described here could be developed/humanized for use as a broad-range agent for prophylactic and therapeutic interventions against diverse strains of influenza A viruses.

Return to Table of Contents