Book of Abstracts

2010 Health Canada Science Forum

Science in the Service of Health

1.10 Qualitative and Quantitative Analyses of Neuraminidase in Influenza Vaccine Preparations Using Antibodies Targeting the Universally Conserved Sequences

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SUMMARY: Influenza vaccines content has yet to be fully standardized due to the lack of appropriate reagents or suitable analytical methods. Simple antibody-based assays were developed which could be of practical value for better vaccine quality control.

OBJECTIVES/BACKGROUND/ISSUE(S): Neuraminidase is a surface protein present in influenza viruses whose functional role in facilitating the release of the virus particles from infected cells has been well characterized. Neuraminidase-induced immune responses are also correlated with protection of humans and animals from influenza. However, the amounts of neuraminidase in current influenza vaccines are yet to be standardized due to the lack of appropriate reagents or a suitable analytical method. Thus, a reliable assay capable of quantifying neuraminidase is desirable for better quality control of influenza vaccines. Our objective was to develop simple antibody-based assays capable of measuring neuraminidase levels in vaccine preparations.

DESIGN/METHODS/DESCRIPTION: Two universally conserved sequences in all influenza A and B neuraminidases were identified by bioinformatics, one at the N-terminus and the other close to the enzymatic active site. Peptides with these conserved amino acid sequences were synthesized and used to immunize rabbits for the production of mono-specific polyclonal and monoclonal antibodies. The specificity of the antibodies was assessed by testing their ability to bind to recombinant neuraminidase protein, fusion proteins consisting of the peptides used as antigens fused to glutathione S-transferase (GST) as well as to the different subtypes of neuraminidase by using several strains of influenza viruses propagated in eggs and cells. The antibodies were then used in immunoassays such as Western blot and slot blot to quantify neuraminidase in influenza vaccines.

OUTPUTS/RESULTS: The antibodies against both epitopes were capable of binding to all 9 subtypes of neuraminidase and demonstrated remarkable specificity against the viral neuraminidase sequences without any cross-reactivity with egg allantoic and cellular proteins. Importantly, subsequent analysis of influenza vaccine samples by Western blot and slot blot assays using the universally reactive antibodies uncovered marked variations in the neuraminidase levels. These samples were from eight manufacturers using the same vaccine seeds and released in accordance with the international standard potency testing. Considerable differences were also observed between lots from the same producer. These results revealed the necessity for neuraminidase quantification in vaccines.

OUTCOMES/CONCLUSIONS: The antibody-based assays reported here could provide a versatile tool in qualitative and quantitative analysis of neuraminidases for better influenza vaccine quality control.