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Review

Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies

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ABSTRACT

Instability of vaccines often emerges as a key challenge during clinical development (lab to clinic) as well as commercial distribution (factory to patient). To yield stable, efficacious vaccine dosage forms for human use, successful formulation strategies must address a combination of interrelated topics including stabilization of antigens, selection of appropriate adjuvants, and development of stability-indicating analytical methods. This review covers key concepts in understanding the causes and mechanisms of vaccine instability including (1) the complex and delicate nature of antigen structures (e.g., viruses, proteins, carbohydrates, protein-carbohydrate conjugates, etc.), (2) use of adjuvants to further enhance immune responses, (3) development of physicochemical and biological assays to assess vaccine integrity and potency, and (4) stabilization strategies to protect vaccine antigens and adjuvants (and their interactions) during storage. Despite these challenges, vaccines can usually be sufficiently stabilized for use as medicines through a combination of formulation approaches combined with maintenance of an efficient cold chain (manufacturing, distribution, storage and administration). Several illustrative case studies are described regarding mechanisms of vaccine instability along with formulation approaches for stabilization within the vaccine cold chain. These include live, attenuated (measles, polio) and inactivated (influenza, polio) viral vaccines as well as recombinant protein (hepatitis B) vaccines.

1. Introduction

The dramatic success of vaccination in improving human and animal health is well established. For example, the US Centers for Disease Control (CDC) ranked vaccination as one of the top ten public health achievements in the United States in the 20th century "… resulted in the eradication of smallpox; elimination of poliomyelitis in the Americas; and control of measles, rubella, tetanus, diphtheria, *Haemophilus influenzae* type b, and other infectious diseases in the United States and other parts of the world." [1]. The past decade (2000–2010) witnessed the development and worldwide regulatory approval of many important new vaccines offering protection against bacterial (meningococcal and pneumococcal) and viral (rotavirus and human papillomavirus) infections. In addition, new vaccine formulations to protect against influenza (live, attenuated vaccine administered nasally) and varicella (for protection against zoster for adults) infections were also successfully developed and approved for use. Furthermore, new combination formulations of more well-established vaccines were commercialized to reduce the complexity of the vaccination schedule and to improve compliance including MMRV (measles, mumps, rubella and varicella), DTaP-HepB-IPV (diphtheria, tetanus toxoid, acellular pertussis, hepatitis B and inactivated poliovirus), and DTwP-HepB-Hib (diphtheria, tetanus toxoid, whole cell pertussis, hepatitis B and Haemophilus influenzae type B) vaccines. This availability of numerous new vaccines, combination vaccines, and improved formulations raises important challenges in terms of procuring and distributing them worldwide [2-4].

Along with these successes in introducing new vaccines to improve public health over the past decade, there have been concomitant major advances in our scientific understanding of the basic biological mechanisms of the human innate and adaptive immune systems as well as the molecular basis by which pathogens cause human disease. Despite these advances, the fulfillment of the

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Abbreviations

ADDC	antibody-dependent cell-mediated cytotoxicity
AFM	atomic force microscopy
ASO3	adjuvant system 03
CCID50	50% cell culture infective dose
D2O	deuterium oxide
DTP	diphtheria, tetanus, and pertussis
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FFA	fluorescence focus assay
FFU	fluorescent focus units
GRAS	generally regarded as safe
GSK	GlaxoSmithKline
HBsAg	Hepatitis B Surface Antigen
HBV	hepatitis B virus
Hib	haemophilus influenzae type B
HIV	human immunodeficiency virus
HPV	human papillomavirus virus
ICH	International Conference on Harmonization
IM	intramuscular
IN	intranasal
IPV	inactivated polio vaccine
LAIV	live attenuated influenza vaccine
mcg	microgram
MDCK	Madin–Darby Canine Kidney
MMR	measles, mumps, and rubella
MMRV	measles, mumps, rubella and varicella
MPL	monophosphoryl lipid A
NA	influenza neuraminidase
NVAC	National Vaccine Advisory Committee
OCC	outside cold chain
OPV	oral polio vaccine
PC	Percutaneous
RSV	respiratory syncytial virus
SC	subcutaneous
SRID	single radial immunodiffusion assay
TEM	transmission electron microscopy
TCID50	median tissue culture infective dose
TIV	trivalent inactivated vaccines
TT	Tetanus Toxoid
VERO	cells derived from kidney of African green monkey
VLP	virus like particle
VVM	vaccine vial monitor
VZV	varicella zoster virus
WHO	World Health Organization

promise of new vaccines is still lacking for many urgently needed, unmet medical conditions such as protecting against infectious diseases (e.g., malaria, HIV, RSV) as well as therapeutic treatments for cancer. In addition, the rapid emergence of new infectious disease threats to public health (e.g., H1N1 pandemic influenza) underscores the importance for rapidly moving from the identification of a new vaccine immunogen in the laboratory to a stable, commercially available vaccine formulation. Clearly, the need to develop, produce and distribute new vaccines to address unmet medical needs remains a high priority for improving public health.

Vaccine formulation development is an important part of the overall development cycle for producing, testing and approving new vaccine candidates. Vaccine formulation can be defined as "converting vaccine antigens to medicines" in which the commercial dosage form not only maintains the potency and stability during manufacturing and storage, but also is designed to be conveniently administered to patients. The modern vaccine formulation development path from the discovery of an immunogen to a usable vaccine includes: (1) physical and chemical characterization of the antigenic component, (2) development of stability-indicating assays including potency, (3) evaluation and optimization of the route of administration and adjuvants (in both animal models and in clinical trials), and (4) formulation design to maximize the candidate vaccine's (antigen and adjuvant) stability, shelf life, and immunogenic potential. A major focus of vaccine formulation development, in many cases, is the enhancement of potency through the use of vaccine adjuvants, since many candidate immunogens fail to transfer from the laboratory to the patient due to suboptimal efficacy in humans. One key approach to increase the success rate for new vaccine candidates is thus to ensure the appropriate formulation in the presence of conventional and/or novel adjuvants. Adjuvants not only promote the rate and extent of an immune response, but can potentially steer the immune response in the desired direction (e.g., humoral vs. cellular immune responses). In fact, a review of the five revolutions in the history of vaccine development [5] predicts the sixth revolution in vaccinology will be the introduction of novel vaccine formulations with novel delivery systems.

The purpose of this review is to raise awareness about the scientific and technical challenges encountered to successfully formulate and stabilize different types of vaccines, both in terms of stability of antigens, adjuvants and their complexes. These efforts are described in the context of maintenance of vaccine potency across the vaccine cold chain, during both clinical development and commercial distribution. Since vaccine stability and potency are defined by the analytical assays used to measure and monitor their physical, chemical and biological integrity, the design and development of accurate, precise and quantifiable analytical methods plays a key role in these stability assessments.

2. Overview of currently available vaccine formulations

A summary of the composition and stability parameters of some representative vaccines commercially available worldwide is provided in Tables 1 and 2 (e.g., FDA approved bacterial and viral vaccines [6], respectively). Based on published reviews [7–11] and publically available information online from various manufacturers and PATH [12], the vaccines listed in Tables 1 and 2 are summarized from the point of view of key pharmaceutical attributes including the vaccine type and manufacturer, formulation dosage form, route of administration, type of adjuvants, and storage considerations (e.g., shelf-life, heat and freeze sensitivities).

One general trend to note from Tables 1 and 2 is that live, attenuated vaccines do not contain adjuvants, but are more heat sensitive to potency loss during storage and distribution. These vaccines contain weakened, attenuated versions of infectious viruses and bacteria that must replicate *in vivo* (and therefore mimic natural infection). Some live, attenuated vaccines are administered orally (e.g., OPV and rotavirus) or nasally (influenza) which mimics the natural route of infection. Many live vaccines are administered by parenteral injection (e.g., IM or SC), yet still provide protection against infections often acquired naturally by other routes (e.g., oral-fecal). In addition, live, attenuated vaccines often require only one or two injections to generate a protective immune response. From a stability perspective, live, attenuated vaccines are often freeze-dried (lyophilized) in the presence of a complex mixture of

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