An alternative method for preparation of pandemic influenza strain-specific antibody for vaccine potency determination

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abstract

The traditional assay used to measure potency of inactivated influenza vaccines is a single-radial immunodiffusion (SRID) assay that utilizes an influenza strain-specific antibody to measure the content of virus hemagglutinin (HA) in the vaccine in comparison to a homologous HA reference antigen. Since timely preparation of potency reagents by regulatory authorities is challenging and always a potential bottleneck in influenza vaccine production, it is extremely important that additional approaches for reagent development be available, particularly in the event of an emerging pandemic influenza virus. An alternative method for preparation of strain-specific antibody that can be used for SRID potency assay is described. The approach does not require the presence or purification of influenza virus, and furthermore, is not limited by the success of the traditional technique of bromelain digestion and purification of virus HA. Multiple mammalian expression vectors, including plasmid and modified vaccinia virus Ankara (MVA) vectors expressing the HAs of two H5N1 influenza viruses and the HA of the recently emerging pandemic H1N1 (2009) virus, were developed. An immunization scheme was designed for the sequential immunization of animals by direct vector injection ollowed by protein booster immunization using influenza HA produced in vitro from MVA vector infection of cells in culture. Each HA antibody was highly specific as shown by hemagglutination inhibition assay and the ability to serve as a capture antibody in ELISA. Importantly, each H5N1 antibody and the pandemic H1N1 (2009) antibody preparation were suitable for use in SRID assays for determining the potency of pandemic influenza virus vaccines. The results demonstrate a feasible approach for addressing one of the potential bottlenecks in inactivated pandemic influenza vaccine production and are particularly important in light of the difficulties in preparation of potency reagent antibody for pandemic H1N1 (2009) virus vaccines.

Measurement of HA Content in Vaccines Using Traditional and Alternative Potency Antibody

- Using recombinant techniques, alternative potency antibody was generated in absence of virus and not dependent upon HA purification

HA content g/ml in vaccine lots determined by SRID using traditional and alternative potency antibody

Vaccine	Potency Antibody[1]	HA (g/ml)					
		A/Vietnam (traditional)	A/Vietnam (alternative)	A/Indonesia (traditional)	A/Indonesia (alternative)	A/California (traditional)	A/California (alternative)
A/Vietnam/1203/2004 (H5N1)							
Lot 1[3]		116±11	100±11	ND[2]	ND	ND	ND
Lot 2		116±20	112±30	124±21	164±4	ND	ND
A/Indonesia/5/2005 (H5N1)							
Lot 1		ND	ND	185±18	303±24	ND	ND
Lot 2		664±86	716±68	664±87	646±50	ND	ND
A/California/7/2/2009 (H5N1)							
Lot 1		ND	ND	ND	ND	79±9	72±2
Lot 2		ND	ND	ND	ND	925±137	1017±34
Lot 3		ND	ND	ND	ND	70±8	70±9

CBER Plays a Major Role in Influenza Vaccine Development

Each year the Center for Biologics Research and Review (CBER) plays a pivotal role in the development of tests that determine if a new influenza vaccine is potent enough to approve for marketing. The same test method is used to measure the potency for all influenza vaccines, but new test components are required for each new influenza vaccine. Although the techniques for making the test components are usually reliable, each influenza virus is different and some viruses have certain characteristics that complicate the development of a vaccine potency test. This can slow development of potency tests for vaccines and thus could delay getting the vaccines to the public. Contingency methods are needed to ensure that test components can be developed in a timely fashion.

In 2009 CBER scientists in the Office of Vaccines Research and Review (OVRR) published details of an alternative technique for making a key component of influenza vaccine potency tests designed to overcome such a problem. FDA has not used this alternative technique to develop a potency test for any vaccine the Agency has approved. However, the technique demonstrates the feasibility of an alternative method for producing potency test components and represents a potential strategy that might be used to overcome a specific obstacle to developing such a test in the future.

Potency Tests Use Antibodies to HA Proteins on Influenza Viruses

Influenza viruses carry on their surface many copies of a hemagglutinin protein (HA). The structures of these HA proteins vary somewhat among the different varieties of influenza viruses. Because healthy immune systems make antibodies against them, HA proteins are the main component of influenza vaccines. The yearly influenza vaccine contains HA proteins from whichever three different influenza viruses are most commonly found circulating throughout the world that year.

A key component of vaccine potency tests are antibodies against each HA protein of the three influenza viruses contained in the vaccine for that year. In order to obtain the anti-HA antibodies used in potency tests, CBER scientists typically remove HA proteins from the influenza viruses using a standard chemical technique. They inject these proteins into sheep, whose immune system makes anti-HA antibodies. CBER harvests these antibodies and uses them to make the potency test for influenza vaccines.

CBER Developed a Backup Technique for Making Potency Tests

The traditional method that CBER uses to remove HA proteins from the virus is designed to preserve the structure of the HA proteins, and this method of removing HA from influenza virus has been very effective with previous influenza vaccines. However, it equires the availability of large amounts of influenza virus in order to purify HA for immunization. In addition, there have been instances where it was difficult to obtain sufficient amounts of HA using this traditional method due to peculiar characteristics of the virus.

CBER scientists led by Jerry P. Weir, Ph.D., director of the Division of Viral Products, used recombinant DNA techniques to develop an alternative technique for immunizing sheep. This strategy does not require the presence or purification of influenza virus and does not require removing the HA proteins from the influenza virus.

First, they injected sheep with DNA coding for HA from either H5N1 (bird flu) or H1N1 (swine flu) viruses. This DNA enabled the animals to produce HA that stimulated their immune system to make antibodies against those HA proteins. The CBER scientists also genetically engineered viral vectors to produce HA, which they used as a booster vaccination for the sheep to further stimulate antibody production. The sheep antibodies against HA worked effectively in tests designed to evaluate commercially produce H1N1 and H5N1 vaccines.

The standard way of preparing potency test components has been successful with previous influenza vaccines. But the new strategy developed at CBER provides an effective backup in case the standard technique does not work well with a ne influenza virus. This work is an example of the critical role CBER research plays in ensuring the safety, purity, potency, and effectiveness of biological products.

The influenza vaccine work at OVRR is centered in the Division of Viral Products (DVP). The DVP laboratory is one of four so-called Essential Regulatory Laboratories around the world that work on behalf of the World Health Organization to facilitate the development and manufacture of safe and effective influenza vaccines made from inactivated viruses (i.e., viruses that are not "live").