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The development and manufacture of influenza vaccines

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The development and manufacture of an Influenza vaccine is unlike any other product in the Vaccine industry because of the need to change composition on a yearly basis. The poor efficacy of Influenza vaccines over the past 2 y in the Northern Hemisphere invites questions on how the vaccines are manufactured and how change in vaccine composition is controlled. The opinion expressed in this commentary is that the risk of not making the correct HA protein is increased by the need to adapt the new seasonal virus for good propagation in embryonated chicken eggs. This adaptation is required because not enough doses can be made in time for the new 'flu season unless productivity is reasonable. This problem is not necessarily solved by going to a cell culture host for virus propagation and that may explain why this more advanced technology approach is not more widely used. A vaccine based on hemagglutinin (HA) protein that does not involve Influenza virus propagation (such as Flublok®) side steps this particular problem. The exact HA sequence can be used as is in the virus. The technology can be run at large scale, already at 2 × 21,000L in Japan, in contrast to eggs where scale-up is by multiplication; the HA product is highly purified and made consistently in the form of rosettes.

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Introduction

Significant challenges and opportunities continue in Vaccine Development and Manufacturing as key unmet medical needs such as HIV, malaria, leishmaniasis and Dengue remain. In addition, new areas are emerging in Therapeutic Vaccines as potential treatments for various cancers. Finally, there is the ongoing challenge of staying one step ahead of the Influenza virus.

In my own case, I led the process development activities for many high quality vaccine product candidates during my more than 20 y at the Merck Research Laboratories. Our principal goal was to develop safe and effective vaccines, gain regulatory approval and succeed in commercialization. A secondary goal was to apply modern Bioprocessing and Bioanalytical technology, much of which was being developed in parallel within the broader Biotechnology community. We experienced a golden period at Merck during which virtually every vaccine candidate in our development pipeline became a successful product with the notable exception being HIV. Successful examples include vaccines providing long-term protection against infections such as HPV, HIB, Rotavirus, Chicken Pox, Shingles, Hepatitis A, Hepatitis B, and Pneumonia.[1]

Soon after leaving Merck in 2009 I became an advisor to Protein Sciences based in Meriden, Connecticut and for the first time in 2010 became part of the Influenza vaccine community. The huge differentiator is that influenza vaccines change on a yearly basis. Nothing in my prior experience would have prepared me to believe that this is possible but clearly the practice is alive and well.

The influenza vaccine community is remarkably well integrated internationally across public health groups, manufacturers, regulatory agencies and delivery to the patient. In the example of the Northern Hemisphere, enough surveillance data must be gathered about emerging influenza viruses so that a prediction can be made in February by the World Health Organization (WHO) for the 3 or 4

strains that should be present in the vaccine for the best protective outcome during the next influenza season. This recommendation is then reviewed by the Vaccine and Related Biological Products Advisory Committee (VRBPAC) one week later for the influenza vaccine composition in the USA.

For a Vaccine Manufacturer this process represents a considerable challenge. From the green light in February, there is very little time to make a seed virus, manufacture and partially purify the surface protein from 3 or 4 different viruses and have many tens of millions of doses filled and ready for release by late summer. To the great credit of the various companies involved this goal is largely met unless there are manufacturing issues. The whole system works reasonably well from the guesswork to predict the most likely strains to having safe and effective vaccine available at an affordable price. Many lives are saved every year!

Process Development and Manufacturing Challenge

What is most surprising of all for an experienced Bioprocess Engineer newly involved in the world of Influenza vaccines is that the manufacturing technology used for making most of the doses is antiquated. Clearly many manufacturers do a very good job with poor technology.

In the USA, a manufacturer will release vaccine but the lot is not available for distribution until released by the FDA. An FDA website is available that summarizes batches released for that year as shown in **Table 1**. So, as of December 3, 2014 we can see that for the 2014/2015 'flu season many individual lots for 10 different products have been released. Thirty-four lots of FluMist® were released of this cold adapted live virus propagated in embryonated chicken eggs. Most of the lots (240 lots) released are for the classical inactivated 'flu vaccine propagated manufactured in embryonated chicken eggs; these lots were made by Novartis, CSL, ID Biomedical Quebec, Sanofi Pasteur and GlaxoSmithKline Biologicals.

Two new vaccines are listed that use innovative manufacturing technology. Eighteen lots of Flucelvax (Novartis) were released; this vaccine is made by propagating influenza viruses in MDCK cells, followed by inactivation and purification. Twelve lots of Flublok (Protein Sciences) were released; Flublok is the first recombinant influenza vaccine based on highly purified HA protein manufactured using the baculovirus expression vector system and insect cell culture technology.[2,3]

There are compelling reasons to move away from egg based manufacturing and the Influenza Vaccine community has been aware of these for many years.[4] In 2005 for example Bruce Gellin, director of the National Vaccine Program at HHS, explained why it's important to end the era of the egg in testimony before a congressional subcommittee on April 12. Recently (Nov17, 2014) Anthony Fauci stated that it was a goal of NIAID "to shift vaccine development from the cumbersome egg-based production to new cell-culture technologies"

At an approximate yield of 1 dose per egg the process design needs to include a steady and reliable supply of chickens able to lay embryonated eggs; this is a challenge at the level of 1 million doses per day manufacturing capacity. This means that at least 1 million eggs per day are being utilized as single use bioreactors; moreover, the embryos need to be 12–14 d old to allow infection with the virus. Purification is difficult, so in general the vaccine is partially purified. Antibiotics and preservatives are often used in the process to maintain adequate asepsis. As a consequence, major companies such as Solvay, Baxter, Sanofi and Novartis for many years have invested heavily in the alternative modern cell culture based technology. Three of these 4 companies have left the 'flu vaccine business.

It is surprising that after all this investment over the past 10 y we face the fact that in the USA, as of December 3, 2014 most influenza vaccine in the USA is made using egg based manufacturing. For other vaccines, cell culture technology has been well established for propagating virus (e.g., Hepatitis A, Rotavirus, JE vaccine, Chicken Pox) so why is 'flu such a challenge? After all well over a billion dollars has been spent collectively by some very capable companies with strong teams to accomplish the goal of moving away from non-scalable egg based manufacturing to modern cell culture based scalable processes. Why is it that

Table 1. Influenza virus vaccine for the 2014–2015 season cumulative 2014/2015 season lot release status (Updated 12/3/2014). Flu vaccine lots that have been released by FDA and are available for distribution by the manufacturers. For information on flu vaccine distribution schedules, please contact the manufacturers directly

Manufacturer	Total Number of Lots Released by FDA
AFLURIA - CSL Limited	52
Fluarix - GlaxoSmithKline Biologicals	6
Fluarix Quadrivalent - GlaxoSmithKline Biologicals	29
Flublok - Protein Sciences Corporation	12
Flucelvax - Novartis Vaccines and Diagnostics, Inc..	18
FluLaval - ID Biomedical Corp. of Quebec	9
FluLaval Quadrivalent - ID Biomedical Corp.	19
FluMist Quadrivalent - MedImmune, LLC	34
Fluvirin - Novartis Vaccines and Diagnostics Limited	55
Fluzone - Sanofi Pasteur, Inc..	38
Fluzone Quadrivalent - Sanofi Pasteur, Inc..	32

the cell culture based vaccines developed by Baxter (Preflucell®), or the Solvay cell culture based 'flu vaccine acquired by Abbott Laboratories have not yet been commercially successful? Why are the doses sold by the leading influenza vaccine company made using egg based manufacturing?

The most probable cause is fold2-. First, egg based manufacturing has been developed over a 70 y time frame and cost of manufacturing is low along with purity; this sets a hurdle. Second, the biggest challenge, which is unique to 'flu, is that the vaccine and thus the seed virus often needs to be changed on an annual basis. As soon as the new virus is declared to be a component of the vaccine a manufacturer has to be able to adapt that virus to the standard cell line. For example, this could be VERO cells and if the virus does not propagate well in those cells it will need to be adapted for better infection of VERO cells. This needs to be done quickly to meet the timeline and the process of propagation needs to be highly productive, also because of the timeline. If its not productive, then not enough doses can be made during the available window. The process of adaptation to a new cell substrate will change the virus and the manufacturer needs to keep this in mind to be sure that it still will provide adequate protection as a vaccine. Recent data from egg based manufacturing illustrate how changes in the strain adapted to growth, in this case propagation in embryonated chicken eggs, had poor vaccine efficacy[5,6]

A simpler solution to this is now available. The virus is surrounded by a lipid containing envelope containing 2 major glycoproteins: HA and neuraminidase. The HA glycoprotein is widely accepted as the critical antigen for vaccine protection as antibodies against HA exhibit neutralizing activity.[7] The HA protein is the component of the virus that keeps changing, thus requiring seasonal changes to the vaccine. Protein Sciences have developed a vaccine, Flublok, based on the HA protein, and data from the clinical studies were sufficiently compelling to support FDA licensure of Flublok for adults older than 18 y old (initially on January 16, 2013 and expanded on October 29, 2014) for the prevention of influenza.[3] The HA component of the vaccine can be changed rapidly as needed on a seasonal basis. This will be described in the next section.

Pandemic Preparedness

The recent emergence of the H7N9 influenza virus in China can serve as an illustration of responsiveness to a real event.

It has long been recognized[4] that egg based manufacturing is not practical for dealing with the emergence of a new avian virus; timelines are too long and the virus is infectious to chickens.

The Cell Culture approach is scalable and potentially fast. The challenges relate to having to handle a dangerous pathogenic virus and to evolve a modified version of this virus. The unknown is the ability of the virus to propagate on the chosen cell line (which could be MDCK or VERO cells). If the productivity is low then the virus needs to be adapted. After adaptation will it still be protective against infection in the inactivated form?

The Protein Sciences Technology provides a good path forward. The genetic code for the HA protein is required and in this example was rapidly available from the internet. HA cDNA can be inserted into the baculovirus used as part of the standard *expres*SF+® insect cells / baculovirus expression system. The Insect Cells are grown in suspension culture under serum free conditions and the HA protein can be purified using the already FDA licensed Universal Process. Using an appropriate facility, vaccine can be made at, for example, the 21,000L scale under GMP within 38 d from getting the DNA sequence.[3] Recent developments have shortened this timeline.

Technology Transfer of Manufacturing

Although the technology is simple, egg based manufacturing is in fact complicated to transfer, let's say to a Developing World country.[8] There are 2 processes; one to make many egg embryos free of avian disease, and this involves a lot of infrastructure, planning and special handling of chickens and the second process is to propagate, purify and inactivate the influenza virus.

The Cell Culture based process has a relatively standard technology. But the virus needs to be highly contained during processing and this requires specialized facilities. Also, adaptation of a new virus to the standard cell substrate is sometimes a major challenge as was described earlier.

The Recombinant approach (Insect cell culture based) for making HA proteins is very similar to manufacturing antibodies using CHO cells. The fact that Biosimilars are now being made in many parts of the world illustrates that this Technology Transfer is practical. In a pandemic situation several of the many already existing CHO based facility for making antibodies could be readily converted to an insect cell culture facility to make HA protein.

Discussion

Various options for manufacturing influenza vaccines are described in this Commentary. Given that there are well established correlates of protection and large amounts of available clinical data, it is clear that HA protein alone is in fact sufficient to form the basis of an effective influenza vaccine. This provides an elegant path forward from a Biochemical Engineering perspective. With appropriate investment of resources the cost of goods can be further greatly reduced for this approach in a very analogous way that the cost of making antibodies using CHO has been reduced.

It is much simpler to manufacture a protein than a virus. In the past the argument was made that it's best to use the whole virus because components like neuraminidase will contribute to providing immunity. But the vaccine this year does not provide good protection presumably because more than 2-thirds of circulating A (H3N2) viruses are antigenically and genetically different (drifted) from the A (H3N2) vaccine component of 2014–15 Northern Hemisphere seasonal influenza vaccines as produced in eggs. (CDC web site Jan 16,2015 (Morbidity and Mortality Weekly Report (*MMWR*)). Another

conclusion that can be drawn from this poor performance is that other components such as neuraminidase do not in fact provide significant immunity. Immunity comes from getting the right HA and the easiest way to make the right HA is to manufacture the protein, not the virus.

Effective protein based vaccines for HPV and Hepatitis B have also been licensed. What is common to these vaccines and Flublok is that the protein antigen is present in the form of a particle. For HPV this is a Virus Like Particle (VLP) and for Influenza (Flublok) the particle is in the form of remarkably uniform rosettes [1–4] The general assumption is that the particle will be more immunogenic than soluble protein. In fact, Flublok does not contain an adjuvant. The recombinant protein approach to influenza vaccine provides an excellent platform for dealing with a pandemic and an example for H7N9 is provided in this commentary. The baculovirus based insect cell expression platform can be used to quickly making a new HA protein as soon as the sequence is published.

An extrapolation can be made to other diseases resulting from virus infection. For example, with Ebola the outer glycoprotein could probably be the basis of an effective vaccine antigen. This combined with a potent adjuvant has a reasonable probability of being a safe and effective vaccine. One general approach to potential pandemics can be devised based on knowing the genetic code of the virus, a determination of which protein antigens and which functional epitopes are likely to be effective as a vaccine, the capability of quickly making the protein in the form of a particle, and the availability of acceptable adjuvants or improved delivery technologies.

It was visionary for BARDA to support the development of protein-based vaccines such as Flublok as an alternative to egg based manufacturing and also as an alternative to working with the virus. All that is needed is the genetic code, and this can be downloaded from the Internet. A powerful argument for this approach is that the HA sequence does not need to be altered in contrast to the virus based manufacturing processes. In theory, this approach should be a more reliable way for making effective influenza vaccine because the new HA can be readily made using the same nucleotide sequence as in the virus.

Disclosure of Potential Conflicts of Interest

Barry C Buckland is a Senior Advisor to Protein Sciences.

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