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FluBlok, a next generation influenza vaccine manufactured in insect cells

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Abstract

FluBlok, a recombinant trivalent hemagglutinin (rHA) vaccine produced in insect cell culture using the baculovirus expression system, provides an attractive alternative to the current egg-based trivalent inactivated influenza vaccine (TIV). Its manufacturing process presents the possibility for safe and expeditious vaccine production. FluBlok contains three times more HA than TIV and does not contain egg-protein or preservatives. The high purity of the antigen enables administration at higher doses without a significant increase in side-effects in human subjects.

The insect cell–baculovirus production technology is particularly suitable for influenza where annual adjustment of the vaccine is required. The baculovirus–insect expression system is generally considered a safe production system, with limited growth potential for adventitious agents. Still regulators question and challenge the safety of this novel cell substrate as FluBlok continues to advance toward product approval. This review provides an overview of cell substrate characterization for *expres*SF cell line used for the manufacturing of FluBlok.

In addition, this review includes an update on the clinical development of FluBlok. The highly purified protein vaccine, administered at three times higher antigen content than TIV, is well tolerated and results in stronger immunogenicity, a long lasting immune response and provides cross-protection against drift influenza viruses.

Keywords: Baculovirus; Cell substrate; Insect cells; Hemagglutinin; Influenza vaccine

1. Introduction

FluBlok, a trivalent recombinant hemagglutinin (rHA) vaccine, is under development by Protein Sciences Corporation. The mechanism of action of FluBlok is the same as that of the licensed egg-grown trivalent inactivated influenza vaccines (TIVs), thereby simplifying the regulatory pathway for product approval. FluBlok is formulated to contain 45 μg of each HA, three times the amount of HA as is contained in TIV. The higher HA content offers the potential to provide cross-protection for which preliminary evidence has been presented, but also the possibility for longer lasting and improved immunogenicity [1–3]. Clinical results suggest that FluBlok may provide superior protection against influenza infection especially in at-risk populations (adults over 65 years, immuno-compromised, etc.) as has been reported for increased antigen concentration of TIV [4,5]. This review discusses the use of a novel cell substrate for the production of hemagglutinin and summarizes some key immunogenicity results from clinical studies that were used to support licensure of FluBlok under the "Accelerated Approval" mechanism in the United States. In addition, the correlation between post-vaccination titer (Day 28) and acquisition of influenza infection is discussed.

Most current influenza vaccines are generated in embryonated hen's eggs. Virions are harvested from the egg allantoic fluid, chemically inactivated and treated with detergent, and either a whole virion preparation is generated, or the HA and neuraminidase (NA) proteins are partially purified to produce split-product, subvirion, or subunit vaccines [6]. Although this system has served well for over 50 years, there are several well-recognized disadvantages to the use of eggs as the substrate for vaccine production. TIVs are standardized to contain 15 micrograms (μg) of each of three HAs, derived from influenza A subtype H1N1, H3N2 and B [7]. Thus, HA, the dominant surface glycoprotein on the influenza virus and

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recognized key antigen in the host response to influenza virus in both natural infection and vaccination, is a logical candidate for recombinant vaccine technology [8].

FluBlok contains HA protein antigens that are derived from the three influenza virus strains, which have been selected for inclusion in the annual influenza vaccine by the WHO and are updated on an annual basis. The three proteins are produced in a non-transformed, non-tumorigenic continuous cell line (*expres*SF+® insect cells) grown in serum-free medium. The cell line is derived from Sf9 cells of the fall armyworm, *Spodoptera frugiperda*. Each of the three recombinant HAs is expressed in this insect cell line using a viral vector (baculovirus *Autographa californica* Nuclear Polyhedrosis Virus).

The HA antigens included in FluBlok are full length proteins containing the transmembrane domain and the HA1 and HA2 regions. The HA proteins form trimeric structures under electron microscopy and are not cleaved in insect cells in the absence of exogenously added proteases (with the exception of HAs containing the highly cleavable sequence of basic amino acids at the cleavage site). Therefore, they are sometimes referred to as rHA0. Since the cleavage site is not known to be involved in the immune response, there should be no significant difference between the immune response to cleaved or uncleaved HA. The individual HAs are extracted from the cells with buffer and detergent and further purified by using a combination of filtration and column chromatography methods. Details on the production and characterization of the rHA are described elsewhere [9,10]. The mechanism of action of this vaccine candidate is expected to be similar to TIV; namely, the induction of HA inhibition (HI) antibodies to prevent influenza infection [11,12].

Manufacturing in insect cells offers a number of advantages over currently licensed influenza vaccines that are produced in embryonated chicken eggs: (i) the influenza rHA antigens are produced using a scaleable, reproducible, and low bioburden fermentation process in insect cells, which results in a consistent, protein-based vaccine with low endotoxin content [1,9]; (ii) selection or adaptation of influenza virus strains for production at high levels in eggs is not required, enabling a good genetic match between the vaccine strains and the disease causing influenza virus strains [1,9]; (iii) the cloning, expression and manufacture of FluBlok can be accomplished within a brief period of time, generally less than two months; and (iv) the manufacture of FluBlok does not require high-level bio-containment facilities, which may result in more rapid production and lower cost of vaccine in the event of the emergence of a new epidemic or pandemic strain of influenza virus; and (v) purification procedures for rHA do not include influenza virus inactivation or organic extraction procedures, thus avoiding possible denaturing effects and additional safety concerns because of residual toxic chemicals in the vaccine [9]. Perhaps most importantly, from a clinical perspective, FluBlok is highly purified and does not contain ovalbumin or other antigenic proteins present in eggs [1,9].

In addition to presenting advantages in manufacturing, as discussed above, insect cells provide safety advantages for the production of biologicals. Insect cells can be grown in the absence of fetal bovine serum and other animal derived ingredients, significantly reducing the chances of introducing an adventitious agent during manufacturing [13–15]. The genetic distance between insects and vertebrates also reduces the likelihood of insect cells serving as a host for vertebrate viruses or the likelihood of vertebrates serving as a host for insect viruses. In fact, many insect viruses described to date exhibit a relatively narrow host range with only a small number of viruses capable of amplifying in both insects and vertebrates [16,17]. These insect viruses along with some tick viruses that can also amplify in vertebrates are informally referred to as arboviruses reflecting their arthropod-borne origin, and have closely co-evolved with the hematophagous arthropods and the vertebrate hosts upon which they feed [17]. The susceptibility of Sf9 cells to arbovirus infection is reported to be very low; with St. Louis encephalitis virus being the only arbovirus tested to date that could produce a persistent, productive and cytopathic infection [18].

The recent progress in using novel animal cell lines as substrates for the production of biologicals has led to the re-evaluation of existing criteria used for evaluating the acceptability of such cell lines. Improvements to existing criteria for determining the acceptability of novel cell substrates as well as development of new criteria have recently been the focus of regulatory agencies [19].

2. Insect cell substrate – *expres*SF+

Protein Sciences uses the baculovirus expression vector system to produce recombinant proteins in the proprietary lepidopteran insect cell line *expres*SF+ (SF+). SF+ cells were derived from the Sf9 cell line, which was first cloned via dilution plating of the mixed population cell line IPLB-Sf-21AE by Cherry and Smith at Texas A&M University (unpublished, 1983). At the time the Sf9 line was developed, the IPLB-Sf-21AE cell line had been in continuous culture since its isolation in 1970 from primary cultures of normal embryonic and ovarian tissues dissected from pupal ovaries of the fall armyworm, *S. frugiperda* [20].

Several characteristics of SF+ cells make them well suited for use as cell substrates for the manufacture of biologicals using the baculovirus expression vector system: (i) SF+ cells are grown in suspension in an inexpensive animal product-free medium to high densities and without clumping; (ii) cultures of SF+ cells are routinely scaled to 450 L under cGMP conditions and maintain a consistent cell doubling time of approximately 18–24 h; (iii) significantly higher yields of recombinant proteins are obtained in SF+ cells as compared to the parental cell line Sf9; (iv) SF+ cells support robust growth of recombinant baculoviruses resulting in high titer stocks of virus, thus allowing for protein expression and virus stock production to be carried out using a single qualified cell line; and (v) these attributes are consistent for over 50 passages allowing for significant flexibility in maintenance of seed stocks during large-scale cGMP production.