Background Summary for the November 16, 2005, VRBPAC Meeting: Use of MDCK Cells for Manufacture of Inactivated Influenza Vaccines

Introduction

Cell culture-grown influenza vaccines have certain advantages over the traditional egg-based vaccines, such as higher virus yields, the potential for more rapid scale-up of vaccine manufacture, the ability to bank and thoroughly characterize cells used in vaccine production, and the potential for adaptation to serum free growth. One cell substrate that is being proposed for use in the US for influenza vaccines is the Madin Darby canine kidney (MDCK) cell line.

Manufacturers of influenza vaccines have taken note of the potential advantages of using MDCK cells in vaccine production. MDCK cells are a continuous (immortal), neoplastic cell line with the potential to become highly tumorigenic. Because no vaccine licensed in the U.S. has been produced in a tumorigenic cell substrate, OVRR would like to update the VRBPAC on the approach it has taken to the regulation of neoplastic cell substrates in general and to review with the Committee its approach to addressing issues associated with cell-substrate tumorigenicity.

This meeting is part of a process that OVRR has undertaken to keep the Committee informed of the regulation of novel cell substrates for vaccine production. Specifically, this meeting will focus on OVRR's approach to the regulation of cell substrates that are highly tumorigenic.

History of OVRR Discussions with the VRBPAC

In 1998, OVRR initiated discussion with the Committee on the potential use of neoplastic cell substrates for vaccine manufacture. At that time, OVRR proposed using a Defined Risks Approach (DRA) to address issues posed by vaccine manufacture in neoplastic cells, and presented it to the Committee for their consideration. The DRA involves:
1. Identifying the possible risk event; 2. Estimating or determining the frequency with which the risk event might occur or has been observed to occur, either in nature or under experimental conditions; 3. Estimating the possible frequency of the risk event per dose of vaccine; 4. Developing and determining the sensitivity (with respect to lower limits of the assay's ability to detect the risk event) of one or more assays that can be use to detect the risk event; 5. Developing and validating one or more processes that can be used to establish a product-specific safety factor at a defined level of risk. The recommendations from that discussion were: 1. OVRR should undertake research to provide the experimental basis for the estimations of risks; 2. Develop a document that describes the approach; 3. Organize an international workshop at which the

issues are presented and discussed in public; and 4. Continue the dialogue with the Committee.

In September 1999, the CBER-sponsored international workshop (*Evolving Scientific and Regulatory Perspectives on Cell Substrates for Vaccine Development*) took place in Rockville, MD. The proceedings were published in Development in Biologicals, volume 106, 2001. The goals of the workshop were to identify concerns and issues associated with neoplastic cells and identify possible approaches to implementing the DRA by ascertaining the levels of risk associated with those issues. Several conclusions were drawn from the presentations and discussions. 1. Because of the multi-factor nature of human carcinogenesis, the risk that cellular components (other than oncogenic viruses) could induce tumor formation was considered low. 2. The risk from adventitious agents was considered greater than from cellular components such as DNA. In this regard, primary cells were considered more of a risk than cell lines. 3. The risk from residual cell-substrate DNA was considered low, although more data were needed on this topic. 4. The possibility that adverse consequences could arise through interactions of the vaccine virus with the cell substrate would be specific to the particular vaccine virus and cell substrate. 5. The potential value of designing cell substrates for the production of specific vaccines was discussed. In general, the approach of immortalizing primary cells by specific mechanisms to produce "designer" cell substrates was met favorably. 6. The issue of whether neoplastic cells had a higher risk of containing a mutation in the PrP gene such that the cells had an increased capacity to propagate the agent of transmissible spongiform encephalopathy (TSE) was discussed. As there was no clear mechanism that could be invoked that would generate *de novo* mutations in the PrP gene, and as there was no obvious selection for mutant PrP proteins, the likelihood of a neoplastic cell developing a mutant PrP was considered to be extremely low. Immediately following this international meeting, OVRR summarized these conclusions to the VRBPAC.

In May 2000, OVRR discussed with the Committee the use of Vero cells for the production of live attenuated virus vaccines. Vero cells are a continuous cell line derived from the kidney of an African green monkey. Like cells from most species (except humans), passage of African green monkey cells in culture can become immortal. The mechanism by which cells "spontaneously" become immortal is unknown but likely involves a number of genetic changes that accumulate over time. Vero cells have the advantage in being permissive to a number of viruses; also, extensive experience with them has failed to reveal the presence of any adventitious agents. These characteristics have resulted in the extensive use of Vero cells both for the testing for adventitious agents and for vaccine production. Early passage Vero cells are not tumorigenic when assessed in immune incompetent rodents, such as the nude mouse or newborn rats treated with anti-thymocyte globulin. However, later passage Vero cells can become tumorigenic, and thus only low passage, non-tumorigenic Vero cells are used for vaccine production. The recommendations from the Committee were: 1.

That the removal of whole cells be assured; and 2. That the level of residual Vero DNA be kept to 10 ng per dose or below. In addition, the Committee concluded there was more of a concern with the use of Vero cells for parenteral vaccines than for vaccines administered by the oral or nasal routes. The Committee also expressed some concern that Vero cells have the capacity to become tumorigenic and asked OVRR to establish a research program to investigate the mechanism by which this occurs to determine if there could be a safety concern.

In July 2001, OVRR asked the Committee to consider the use of adenovirus type 5 (Ad5)-transformed cells for vaccine production. These cells are necessary to manufacture defective Ad5 vectors, since they contain the E1 genes (E1A and E1B) necessary to complement the E1-defective virus vectors. Because Ad5 vectors propagated on the original production cell line, 293, were found to contain replication-competent adenovirus (RCA), which was generated by recombination with the E1 genes present in the complementing cell, a new cell line was developed such that the only Ad5 sequences present were those necessary for complementation, and recombination to generate RCA was eliminated. The new cell line was the PER.C6 cell line, which was established in the Netherlands from fetal retinoblasts immortalized by the Ad5 E1A and E1B genes driven by the cellular promoter from the phosphoglycerate kinase gene. It was found that PER.C6 cells were weakly tumorigenic (see below), forming tumors at 10^7 cells per nude mouse, a level similar to that found with 293 cells.

Because the use of PER.C6 cells for vaccine manufacture would be the first time a live viral vaccine produced in a tumorigenic cell substrate would be permitted by CBER to be used in humans, OVRR brought this topic to the Committee for their consideration. While adventitious agents and residual cell-substrate DNA are potential concerns with all novel cell substrates, there may be a heightened concern when the cell substrate is tumorigenic or derived from a tumor. The potential risk of adventitious agent contamination (including TSE contamination, since PER.C6 cells are neural derived), residual cell-substrate DNA, and whole cells were discussed. The sponsor provided documentation on the adventitious agent testing, calculations as to the risk of TSE transmission, the level of residual DNA, and the removal of whole cells. The committee discussed factors that could mitigate concern about these cells, including the relevance of the known mechanism of transformation and the requirement for approximately 10^7 cells to produce tumors in immunosuppressed animals. The Committee also discussed the importance of minimizing steps toward oncogenesis in vaccine recipients (such as initiating events), even when oncogenesis was not a direct outcome of vaccine components. The Committee agreed that PER.C6 cells represented an improvement over 293 cells with respect to RCA generation and were satisfied that the sponsor had addressed the issues of adventitious agents and residual cell-substrate DNA. The Committee also endorsed OVRR's general approach to evaluating these cells, including the evaluation of cell lysates and DNA from PER.C6 cells for their oncogenic potential in animals, even though there were no validated assays available.

The Current Meeting

The consideration of Madin Darby canine kidney (MDCK) cells for the manufacture of inactivated influenza vaccines has prompted this meeting. While some lines of MDCK cells are not tumorigenic, others are highly tumorigenic. Thus, one goal of this meeting is for the Committee to comment on our modified DRA as applied to highly tumorigenic cell substrates.

As stated above, the main safety concerns with the use of tumorigenic cells are the potential presence of adventitious agents, particularly of unrecognized oncogenic viruses, and the amount and the form of the residual DNA present per vaccine dose. A key and unresolved scientific question is whether and how the degree of tumorigenicity of a cell substrate should influence the testing algorithm for the determination of its suitability for use in vaccine production.

Tumorigenicity Testing (Dr Andrew Lewis)

For over 40 years, there was a proscription on the use of tumorigenic cells for vaccine production due to concerns that products manufactured in these types of cells could transfer cancer to a vaccine recipient. Whether or not these concerns are scientifically justified cannot yet be determined. Nevertheless, there is a recognized perception that the use of neoplastic cell substrates for vaccine manufacture poses risks for vaccine safety, and these risks are enhanced the more tumorigenic the production cells are. Therefore, because it is critical for the public health to retain trust in vaccine safety, regulatory authorities need to address these risks and the attendant concerns over safety if vaccines are to be manufactured in tumorigenic cells.

Tumorigenicity is the property of a cell that allows it to form a tumor in an experimental animal, usually the nude mouse, while oncogenicity is an activity of an agent (usually a virus) or cellular component (such as DNA) that is able to transform cells of that animal into neoplastic cells that can form a tumor. These definitions have practical consequences, since the species of the tumor cells that occur in a tumorigenicity assay will be that of the donor cell, while the species of the tumor cells that arise in an oncogenicity assay is that of the host. Before 2000, the specific requirements for tumorigenicity testing were single-dose, relatively short-term assays. Such assays are limited both in their capacity to determine whether a line of neoplastic cells expresses a tumorigenic phenotype and the degree of aggressiveness (*i.e*., weakly *versus* highly tumorigenic) of the tumorigenic phenotype. The most comprehensive method of obtaining tumorigenicity data is to evaluate quantitatively, in a dose-response assay with longer observations times, the capacity of the cells to form tumors. These types of assays have revealed that different cells have differing capacities to form tumors: some cells are weakly tumorigenic (*i.e*., requiring large numbers of cells – 10^6 or more – to produce a tumor), while others are highly tumorigenic (*i.e*.,

requiring as few as 1 to 100 cells to produce a tumor). At present, the mechanisms for the variable expression of the tumorigenic phenotype have not been delineated. Until they are, it will not possible to determine whether the use of a cell substrate that is strongly tumorigenic poses more of a risk than one that is weakly tumorigenic. In addition, determining the dose-response relationships associated with tumor formation can be used to enhance vaccine safety by revealing the presence of unrecognized adventitious agents.

Enhanced testing and characterization of the cell substrate is recommended for tumorigenic cells. Therefore, assays more comprehensive than single-dose, short-term assays are required to determine the level of tumorigenicity of a neoplastic cell substrate. OVRR is currently recommending testing of multiple doses of cells (10^7, 10^5, 10^3, and 10^1) in adult nude mice, extending the observation period of the test animals up to 4 to 5 months, and identifying the species of the cells forming the tumors.

Adventitious Agent Testing (Dr Arifa Khan)

Testing for adventitious agents in cell banks and virus seeds involves extensive and overlapping *in vitro* and *in vivo* testing designed to encompass the detection of different classes of viruses. These tests have served the public well over the years, as evidenced by the good safety record of vaccines. However, as new viruses are identified, the testing has evolved and expanded over the years to take advantage of new approaches and novel assays. The most significant addition to adventitious agent testing is the use of PCR methods. These include the development of assays for the detection of specific viruses (DNA and RNA) as well as broadly reactive PCR-based assays, such as the sensitive product-enhanced reverse transcriptase (PERT) assay for the detection of all retroviruses and generic PCR assays for the detection of virus families. The use of tumorigenic cell substrates in vaccines has added concerns regarding the possible presence of novel viruses, especially oncogenic viruses, and therefore broad detection assays that have the potential to detect unknown viruses are needed. These include treatment of the cell substrate with virus inducers followed by general detection methods (*e.g.*, PERT assay, TEM, generic PCR) for evaluating the presence of latent or endogenous viruses and the inoculation of lysates prepared from the cell substrate into newborn mice, newborn rats, and newborn hamsters for detection of oncogenic viruses. Although neither type of assay (virus induction or injection of cell lysate) has been validated, both approaches have been demonstrated to detect viruses. Another approach that is applicable in the case of inactivated vaccines is to determine the amount of viral clearance (removal and/or inactivation) due to the manufacturing process. Spiking studies using viruses with different properties that are expected to influence their removal and/or inactivation are generally used to determine the level of removal and/or inactivation during the various steps in production, with subsequent calculation of the total clearance during manufacture. For highly

tumorigenic cells with negative adventitious agent test results, showing clearance of appropriate model viruses should provide additional assurance of safety.

Regulatory Issues Associated with Residual Cell-Substrate DNA (Dr Keith Peden)

Vaccines and other biological products manufactured in cells contain contaminating residual DNA derived from that production cell substrate, with the amount and form of this DNA depending mainly on the type of vaccine. The potential risk of this cellular DNA has been debated for over 40 years without resolution. Opinions on residual DNA have varied from it being considered an inert contaminant, and thus its presence should not be deemed to be a risk to vaccine recipients, to it being considered an important risk factor, particularly for vaccines manufactured in tumorigenic cell substrates. Because DNA has demonstrable biological activity, OVRR considers that DNA should not be considered an inert contaminant. The activities that DNA can transfer are oncogenic activity and an infectivity activity. DNA oncogenicity is the capacity of DNA to induce a tumor in an animal, while DNA infectivity would result if the cell-substrate DNA contains the genome of an infectious virus and this genome, when transferred to a human vaccine recipient, could result in the establishment of an infection. The major risk for residual cell-substrate DNA has generally been considered to be due to the oncogenicity of the DNA, although DNA infectivity may be more of a risk. Our approach is to develop assays to quantify the particular biological activities of DNA, determine the amount of activity in a given quantity of DNA, and use these values to estimate risk. In addition, the efficiency of methods to eliminate the activity, such as by chemical inactivation or enzymatic digestion, can be quantified using the same assays and thus the level of safety from the clearance of DNA can be estimated. An *in vitro* DNA infectivity assay can detect at least 10^6-fold less DNA than an *in vivo* DNA oncogenicity assay, and thus we are using the *in vitro* infectivity assay to estimate the amount of clearance of DNA that can be achieved using chemical or enzymatic methods. Because estimates of safety are based on the sensitive *in vitro* DNA infectivity assay, this represents a worst case. Using such assays, clearance levels (or safety factors) of infectivity of $>10^7$ can be achieved by inactivation or digestion with a DNA level of 10 ng per dose. With such safety factors, the risk of an infectious event can be reduced to acceptable levels.

Concluding Remarks

The introduction of highly tumorigenic cells to influenza vaccine manufacture could yield significant benefits. Although there is a perception that highly tumorigenic cells may carry greater risks than less tumorigenic cells, we are proposing that such risks can be mitigated by careful testing of the cells, validation of the production process for its capacity to remove adventitious agents, and limitation of residual DNA in the final product. OVRR would like the committee to discuss (1) the use of MDCK cells, including those that are highly

tumorigenic, in manufacture of inactivated influenza vaccines, (2) OVRR's approach to evaluate the safety of highly tumorigenic cells, and (3) any additional steps OVRR should take to address issues associated with the use of MDCK cells or neoplastic cell substrates.