An overview of viral and viral-like agents in cell culture systems

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Abstract

The first recognition of seriously contaminated biological products occurred almost 100 years ago. More recently, in the second half of this century, the potential for contamination by viral and viral-like agents became obvious when the SV-40 virus was identified as an endogenous agent in the cell cultures used to produce polio vaccines. The history of major contamination incidents is reviewed, and current issues are discussed.

Introduction

The historical background of viral and viral-like agents that have contaminated biological products for human use can help to put into context some of the data and issues that will be presented in the papers that follow. The issue of microbial agents – both real and hypothetical – as contaminants of biological products is a very old one. Transmissible spongiform encephalopathies are the latest, and perhaps the most challenging, in a series that began almost 100 years ago. Whether there are lessons to be learned from the past that can be applied to contemporary issues is, of course, the big question which will be addressed at the end of this paper.

From the very beginning, the regulation of biological products in the U.S. and elsewhere has evolved in response to crises. It also has relied heavily on science and scientists in the development of policy and decisions in response to those crises. The first incident involving a biological product which attracted federal interest in the U.S. occurred at the beginning of this century during a diphtheria epidemic in St. Louis in which 14 children inoculated with a diphtheria antitoxin died – not of diphtheria, but of a tetanus contaminant in the antiserum (Report, 1901). While that incident involved a bacterial contaminant, it is relevant to the issue of viral and viral-like agents because it resulted in the 1902 Federal Virus Toxin Act,

much of which still remains intact as part of the Public Health Service legislation that FDA uses to regulate most biological products.

No similar incidents occurred until 1955 when the prospects seemed bright for controlling poliomyelitis with the new Salk vaccine produced in primary Rhesus monkey kidney cells. In that same year, the potential for creating a biological disaster of major proportions in national and global immunization programs was dramatized by a case, commonly referred to as the Cutter incident, in which over 260 children contracted polio after receiving the Salk vaccine of which 10 died because of the incomplete inactivation of the wild-type poliovirus used to produce the vaccine. While this was not, strictly speaking, an endogenous or exogenous contaminant of the product, that tragedy re-emphasized the necessity for maintaining strict control of biological products by the manufacturer and the national regulatory agency.

Viral agents

Subsequently, the first *viral* contaminant of potential major public health significance in a biological product was identified. The virus was SV40 and the products were again inactivated polio vaccines. SV40 is an endogenous virus of Rhesus monkeys and was a frequent contaminant of primary Rhesus monkey kidney

cell cultures used to produce polio vaccines (Girardi, 1962). The public health issues revolved around the fact that SV40 was shown to be tumorigenic in hamsters. There was therefore justifiable concern that the SV40 contaminant in polio vaccines might lead to cancer in the vaccinated children. That concern was heightened with the finding that SV40 could transform normal human diploid fibroblasts, WI-38, *in vitro*.

There were two major responses to the SV40 incident. First, the National Cancer Institute funded a long-term follow-up study of a cohort of those children who had received SV-40 along with the polio vaccine. After 20 yr, there was no evidence of an increased frequency of cancer (Mortimer, 1981). A more recent 30-year followup study confirmed the conclusions of previous reports that there is not a significant correlation between SV40 exposure during the neonatal period and certain cancers (Strickler, 1998). Second, the cell substrate was switched from Rhesus to African Green monkey kidney because SV-40 is not an endogenous virus for that species.

Nevertheless, the issue of endogenous viruses in nonhuman primates as potential contaminants continued to swirl as more and more latent viruses were identified. The potential for serious consequences was accentuated in the 1970s with the deaths of a number of persons who came into direct or indirect contact with African monkeys that were infected with what became known as the Marburg virus (Gear, 1975).

Two solutions to the problems associated with using primary cells as substrates for the production of biologicals were advanced. One was to develop closed and carefully monitored colonies of animals, and in some cases specific pathogen-free colonies, from which primary cell cultures could be derived. The other approach was put forward by Dr. Hayflick and his colleagues (Hayflick, 1961). They developed an alternative to primary monkey kidney cells, namely, the WI-38 human fibroblast cell line which has normal phenotypic characteristics, such as a finite life, a diploid chromosomal constitution, and a lack of tumorigenic potential. In addition, a variety of tests showed no evidence of viral contamination. However, WI-38 was slow to be accepted in the U.S., primarily because of Dr. Albert Sabin's position that they might contain a latent human leukemia virus for which there were no tests (Sabin, 1969). Obviously, if you don't know how to test for an agent that *might* be present, about all one can do is provide as much indirect safety evidence as possible. Eventually, that approach worked, and products derived from WI-38 were ap-

proved by FDA, but long after they had been approved in Europe. In retrospect, Dr. Sabin was correct to have been concerned about human leukemia agents since they were discovered years later. However, he had focussed on the wrong cell type.

The next viral contamination episode came in the 1970s when we discovered bacteriophages in bovine sera and in live viral vaccines that had been derived from cell culture systems in which bovine sera had been used to propagate the cells (Chu et al., 1973; Petricciani et al., 1973). The finding of phages in those biological products had more regulatory implications than public health significance, since phages were simply markers of prior bacterial contamination in the bovine sera, and did not pose a health risk. That discovery, however, focused attention once again on the issue of controlling the quality of materials used in the manufacture of biological products.

Nevertheless, FDA regulations clearly prohibited the marketing of products with a live viral contaminant. A benefit risk analysis led to the conclusion that it would be better to allow vaccines on the market with small amounts of phages in them than to remove those products from the market for some extended period of time while phage-free vaccines were manufactured. The fix, obviously, was to change the FDA regulations. In what is probably the record for a fast-track publication of a regulation, FDA allowed what was termed 'unavoidable bacteriophage' in live viral vaccines while steps were taken to produce bovine sera that were phage-free.

World Health Organization (WHO)

In the mid-1980s, a WHO expert group reviewed the entire area of cell substrates, and made it clear that viral and viral-like contamination was the major concern, and that the manufacturing process for biological products must be shown to eliminate any potential contaminating agents pathogenic for humans, taking into account the type of cell being used as well as the raw materials used in production (Report, 1987).

When the Study Group examined this area, they divided cells into three risk categories with respect to their potential for carrying infectious agents *pathogenic* for human beings, namely, low, medium, and high. In making these risk distinctions, they noted that human diploid cells had been used successfully for over 20 yr and that there was no evidence to support Dr. Sabin's concern about a latent human oncogenic

agent. Similarly, even though avian tissues and cells are well-known to harbor viruses, there is no evidence for transmission of disease to human beings by products from these sources. For example, large quantities of yellow fever, measles, and live influenza vaccines have been produced for many years in eggs that contain avian leukosis viruses, but there is no evidence that these products have caused leukemia or lymphoma during their long history of use for human immunization. However, it is important to note that in 1942, approximately 50 000 U.S. military personnel developed hepatitis B from Yellow Fever vaccine due to contaminated human serum which was used as a stabilizer in the vaccine.

In considering mammalian cells, the WHO Study Group suggested that medium risk is associated with cells derived from non-hematogenous cells. Here, they recognized that rodent cells, like avian cells, are well-known to contain viruses or virus-like structures that are visible on electron microscopy. But products have been produced from such cells without any evidence of transmitting infectious diseases to humans. For example, primary hamster kidney cells have been used to produce rabies vaccine, and Chinese hamster ovary cells have been used extensively to manufacture recombinant DNA products.

And finally, the WHO group concluded that blood and bone marrow cells of human and nonhuman primate origin were high risk based on clear evidence that such human cells frequently transmit infectious diseases. Similarly, there was heightened concern for the potential of transmitting diseases by hybridomas where one partner was of human or nonhuman primate origin.

The primary reason the Study Group viewed viral or viral-like contaminants as the most significant issue from a public health and safety point of view is because they have been the single most important risk in the past, if one looks at the history of actual transmission of infectious diseases by biological products. It is strikingly evident that *primary tissues* – especially human – and *primary cells* have been the source of several problems that occurred in the past. Specifically, as already mentioned, polio vaccines carried SV-40 from primary Rhesus monkey kidney cells. Fortunately, there is no evidence to suggest that the children who received SV-40 in polio vaccines were at greater risk for cancer than those who did not get SV-40 (Strickler, 1998).

Human Plasma Protein Fraction (PPF) from one producer transmitted hepatitis B in the 1970s, prob-

ably as the result of a manufacturing flaw, and some deaths occurred. Human corneas derived from a cadaver transmitted rabies after they were transplanted because the medical history of the cadaver was ignored. Similarly, growth hormone derived from the pituitaries of human cadavers led to the development of Creutzfeld-Jacob disease (CJD) in many of the children who received that product. In addition, dura mater transplanted from cadavers has transmitted CJD. Factor VIII (Antihemophilia Factor) and Factor IX Complex derived from human plasma were licensed in the U.S. in the 1960s. Because neither tests for donor screening nor virus inactivation procedures for these products were available, they transmitted hepatitis viruses. When HIV entered the plasma supply in the late 1970s or early 1980s, it also was transmitted to hemophilia patients, with over 4000 of them in the U.S. having developed AIDS since that time. Currently, we are worried about: 1) the agent responsible for bovine spongiform encephalopathy as a contaminant of beef, especially in Europe; 2) prions as a potential contaminant of blood and blood products; and 3) retroviruses as potential pathogens in transplants of organs from various species to humans.

In contrast to these examples of actual transmission of disease, or concern about possible transmission, cell lines and raw materials can be tested extensively to rule out the presence of potential pathogens for humans. The ability to pre-test cell lines – whether diploid or continuous – is the single most significant factor that has resulted in the *absence* of any documented case of infectious disease transmission by *any* product of which I am aware that was derived from well-characterized and tested cell substrates.

The most recent actions related to potential viral contaminants in biological products occurred in 1996 when the WHO Expert Committee on Biological Standardization and the International Conference on Harmonization revised their respective guidance documents for both diploid cell and continuous cell lines. These updated recommendations recognize the evolution in thinking about cell substrates that has taken place over the past 30 yr, as well as the technology that is currently available, and they emphasize state-of-the-art tests for viruses endogenous to the cells of the species being used for production. And, of course, improved tests also are used to detect *exogenous* contamination of both the raw materials and the product. For example, if a new human cell line were proposed as the cell substrate for various products, and since we know quite a bit about human endogenous viruses, the

polymerase chain reaction could be used in the characterization of the cell line to test for potential latent agents such as CMV, EBV, HTLV-1 and 2, HIV-1 and 2, and hepatitis A, B, and C.

Summary and conclusions

That brings us up to the present, and I think it's fair to ask what we have learned in the past century that could help us in facing the future when we consider the risk of products transmitting infectious viruses and viral-like agents. In a sense, we have been both frightened and reassured. Extraneous agents have, on a few occasions, been administered inadvertently to thousands of recipients of biological products. On balance, however, considering the hundreds of millions, if not billions, of doses and exposures that have occurred during this century, it is remarkable that there have been relatively few problems. However, we need only to think back to the early 1980s when HIV first appeared, or more recently to the difficult issues that prions present, to understand that, as Dr. Fred Robbins often pointed out, we should be prepared for surprises and not be deluded into thinking that we have all the answers or that everything is under perfect control.

If we look at the history of the *actual* transmission of infectious diseases by biological products, there are several points that emerge. First, problems are almost inevitable when strict control of the manufacturing process (including design, validation, and routine execution) or of the starting materials is not maintained, and when up-to-date technology is not applied. Second, a safe product can be made unsafe by the addition of a contaminated excipient (diluent, stabilizer, etc.). Third, clearly the biggest risk of the past has been associated with human agents that have contaminated primary human cells and tissues. The lesson here is that if it is possible to avoid using primary human material, it is safer and wiser to do so. Obviously, that is easier said than done, especially if one has no other

choice, at least for the present, such as with whole blood and many blood derivatives. The final point that becomes obvious from past experience is that conventional wisdom does not necessarily lead us down the right path. For example, the initial anxiety and apprehension concerning the safety of human diploid cells as well as the bias towards using primary tissues and cells did not prove to be well-founded. As we move into the future, we should look carefully at these experiences of the past to guide us in making appropriate decisions about risk.

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