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Introduction to Adventitious Agent Issues

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Public confidence in vaccine safety

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Maximal vaccine benefit

Adventitious Agent

An infectious agent that is extraneous to the product

Goal: Final products should not contain adventitious agents

OVRR approach to adventitious agent issues

- Identify potential issues, including theoretical ones
- Discuss issues in public
- Make decisions based on the best available science
- Ensure that potential issues are known to research subjects

Potential adventitious agents to be considered today

- Transmissible spongiform encephalopathy (TSE) agents
- Viruses

Adventitious agents: Examples

Product	Agent
Yellow fever vaccine	Hepatitis B Virus, ALV
Measles vaccine	Pestivirus
Blood products	Hepatitis viruses, HIV
Urokinase	Reovirus
Growth hormone & Dura mater grafts	CJD agent
Interferon.3	MVM
Polio & adenovirus Vaccines	SV40

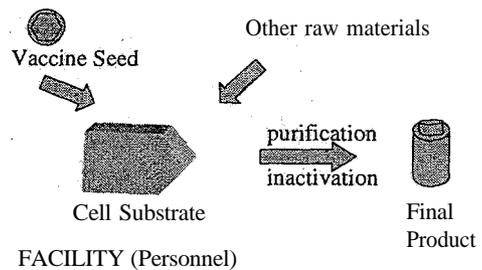
SV40 in vaccines

- Millions received SV40-contaminated pRhMK-produced polio and adenovirus vaccines in the late 50s and early 60s
- Cell supernatants caused tumors in laboratory animals and CPE in pCMK cells
- Vaccine seeds were treated with anti-SV40 neutralizing antibodies in the early 1960s
- Epidemiological studies suggest no adverse sequelae to vaccinated children
- SV40 DNA has been detected in some human malignancies by PCR

SV40: lessons learned

- Value of ensuring that products are free of adventitious agents
- Importance of ensuring freedom of oncogenic agents, especially for vaccines given to children

Viral Vaccine Production



Adventitious agent testing principles

- Should consider issues specific to material in question
- Value of quantitative validation
 - High sensitivity assays include amplification step
 - Controls
- Where possible, should use tests that have the potential to detect unsuspected agents

Creating a quantitative framework for decision-making

- Need to estimate pre-test probability of a problem
- Need to consider number of doses (or dose-equivalents) that can be tested
- Need to understand sensitivity of assays
- Need to consider safety margins

Factors that could influence adventitious agent risk

- Species
- Cell type or tissue of origin
 - previous exposures
 - fetal vs. adult origin
 - tumor association
 - knowledge of transforming event
 - ability to bank cells
- Maintenance or passage history

Vaccine cell substrates

- Whole animals
- Primary cells
- Diploid cell strains
- Neoplastic cell lines

Advantages of neoplastic cell substrates

- Host range
- Cell banking
- Serum-free growth
- Can express complementing genes

Issues associated with neoplastic cell substrates and oncogenic viruses

- Potential that an oncogenic virus was involved in the cell line's neoplastic transformation
- Some of these cell lines are more likely to have uncertain histories
- Potential severe consequences that are difficult to evaluate in short-term clinical studies

Testing for adventitious viruses

Test	Amplification	Potential to detect the unsuspected
Tissue culture (TC)	+	+
Egg inoculation	+	+
Animal inoculation		
-death	+	+
-weight loss	+	+
Animal Ab production	+	-
PCR-based RT (PBRT)	+	+
TC + PBRT	+	+
Specific PCR	+	-
Electron Microscopy	-	++

PCR vs. Biological Assays

- PCR
 - More sensitive for small samples with low residual DNA
 - Very specific
 - works independently of growth characteristics
 - May not represent live virus
- Biological assays
 - *More sensitive-for large samples
 - Greater potential to detect the unknown
 - Requires growth in a specific system
 - More relevant endpoint (e.g., only way to detect oncogenicity)

Methods used to discover viruses

- . Animal inoculation
- . Tissue culture
- . Electron Microscopy
- . Molecular methods

Some tumor viruses discovered using animal assays of cell lysates or supernatants

- Retroviruses
 - Rous sarcoma virus (1911)
 - Feline leukemia virus (1964)
- Poxviruses
 - Rabbit fibroma (1932)
- Papovaviruses
 - Rabbit papilloma virus (1933)
 - Polyomavirus (1953-57)
 - SV40 (1960-62)
- Adenoviruses
 - Ad 12 (1962)

Viral induction of tumors in animal assays

VIRUS	H	M	R
Rous & murine sarcoma viruses	■	■	■
Murine leukemia viruses	■	■	■
Polyoma virus	■	■	■
SV40, BK, JC viruses	■	■	■
Adenovirus 12, 18, 31	■	■	■
Adenovirus 9	■	■	■
SA7 (AGMK adenovirus)	■	■	■
CELO (chicken adenovirus)	■	■	■
Human herpesviruses and papillomaviruses	■	■	■

Animal tests for oncogenic viruses

- . Could be used in cases where additional confidence that a **product** is free of adventitious oncogenic **viruses** is desired
- . Many tumor viruses are &l-associated
- . Inoculating 2 animals models with cell-free lysates of cell substrates, followed by observation for 5-6 months, would lead to maximum sensitivity

In vivo testing of cell substrates for oncogenic adventitious viruses

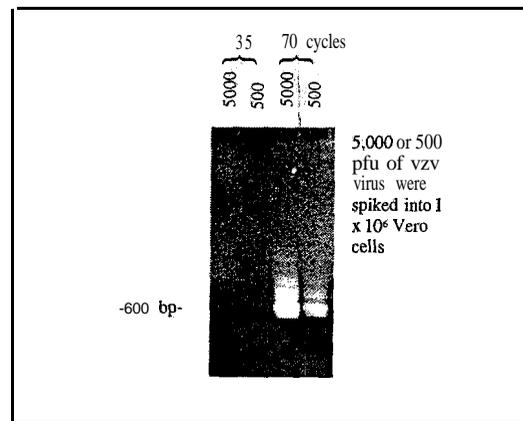
- . Value for ensuring product consistency
- . Best for ensuring absence of potential viral interactions
- . Required to apply principles of clearance
- . Lysate vs. supernatant

Issues with using final product for animal oncogenic adventitious virus testing

- . Potential for interference
 - Cell killing
 - Inflammatory response
 - Effect on apoptosis (especially Et, E4+ viruses)
- . Testing of final product would give assurance that the vector itself is non-oncogenic

New broadly-specific molecular approaches to virus detection

- Use consensus PCR primers to detect related viruses
- Molecular subtraction
- Non-specifically amplify viral nucleic acids



Potential benefit of the product

- Consider the intended recipient
- Some risks from viral adventitious agents are theoretic & **and** must be placed into the context of the benefit of the product

Potential approach to TSE testing of Ad5 transformed human "Designer" Cell Substrates for vaccine production

- Consider
 - Cell type & potential exposures to BSE
- Tests to consider
 - Sequence PrP-encoding gene
 - Western blot
 - Add newer, more sensitive tests as they become available
- Informed consent, investigator brochure

Potential approach to virus testing of Ad5 transformed human "Designer" Cell Substrates for vaccine production

- Perform standard testing, including extensive tissue culture and electron microscopy
- Ensure tests would detect **any** agents based on
 - fetal origin of cells
 - cell type
 - cell history
- Although mechanism of transformation is likely Ad5 genes, do extensive testing for potential oncogenic adventitious agents
 - Cell lysate oncogenicity testing
 - Other tests as they become available
- Informed consent, investigator brochure

TSE and Cell Substrates

- Issue of PrP (PRNP) genotype in cell donor
- Consequences of exposure to serum from countries where BSE or the risk of BSE exists
- Other factors that could increase risk of TSE infection (?PrP expression levels, neuronal or retinal origin of cells)

TSE and Neoplastic Cells

- Potential effect of genomic instability on normal PrP gene
- Potential role of apoptosis in preventing TSE infection

Current approaches to TSE issues

- Where possible, determine family & medical history of cell donor with respect to TSE risk factors.
- Sequence PrP (PRNP) gene
- Perform Western blot for the presence of protease resistant PrP.
- Determine if exposure to FBS from countries with BSE could have occurred.
- If possible exposure to FBS of unknown origin has occurred, perform risk assessment based on dilution factor, assuming that cells cannot support replication of BSE agent.

Evolving approaches to TSE issues

- If possible exposure to FBS of unknown origin has occurred, document that cells can not support replication of BSE agent.
- Evaluate level of PrP expression.
- Evaluate for the presence of infectious TSE agent by animal inoculation.
- Once new assays for detection of TSE agents become available, introduce them for cell substrate testing as soon as feasible.

OVRR approach to TSE issues

- Use existing technically feasible strategies to evaluate cells
- informed consent & investigator brochure
- Present issues to VRBPAC for initial discussion
- Present issues to TSE advisory committee for more comprehensive discussion

Adventitious Agent Testing of Neoplastic Cell Substrates

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