

OPEN SESSION

MAY 16, 2001

### Quantitative Assessment of the Risks of Residual Cell-Substrate DNA

Keith Peden  
Division of Viral Products  
Office of Vaccines Research and Review  
CB-ER

### WHO Requirements for Residual Cell-Substrate DNA Levels

- . WHO Study Group, Geneva, 1986  
Residual DNA from continuous cell lines: 0.1 ng or less
- . WHO Expert Committee on Biological Standardization, 1998  
Residual DNA from continuous cell lines: 10 ng or less

### Types of Perceived Risks Associated with Residual Cell-Substrate DNA,

Oncogenic Risk

Infectivity

Risk

### Proposed Mechanisms of Oncogenic Risk by Cell-Substrate DNA

- . Introduction of a Dominant Activated Oncogene.
- . Insertional Mutagenesis
  - Disruption of a Cellular Tumor Suppressor Gene
  - Activation of a Cellular Dominant Oncogene (Promoter/Enhancer Insertion)
- . DNA Methylation Pattern Changes Following Integration

### Potential Risk of Infectivity Following Inoculation of Cell-Substrate DNA

- . Cell-substrate DNA may encode an infectious genome
  - DNA virus (e.g., polyomavirus, herpesvirus, papillomavirus)
  - Provirus of a retrovirus (e.g., HIV, HTLV)

### Nature of DNA Inoculum

- . Linear *versus* Circular
- . Single-Stranded *versus* Double-Stranded
- . State of DNA - Free *versus* Chromatin
- . Size Distribution of DNA

### Routes of DNA Inoculation

- Intramuscular (i.m.)
- Intradermal (i.d.)
- Subcutaneous (s.c.)
- Intranasal (i.n.)
- Oral
- [Intraperitoneal (i.p.)]

### Assumptions for DNA Activity (1)

- For a given DNA, the level of the response of a cell to that DNA is proportional to the amount of that DNA.
- The activity of a gene integrated in chromosomal DNA or as part of plasmid or phage vector is equivalent or lower.
- The amount of uptake of a given gene by a cell and the expression of this gene in the cell is related to the concentration of the gene in the DNA.

### Assumptions for DNA Activity (2)

- Because a single-copy gene is represented at approximately one millionth of the haploid mammalian genome, the amount of DNA corresponding to a 'single-copy gene is  $10^6$ -fold less abundant for equivalent amounts of cellular DNA compared with a plasmid DNA with the same gene.

Le., for the uptake and expression of a gene in mammalian genomic DNA equivalent to 1  $\mu\text{g}$  of plasmid DNA,  $1 \times 10^6$   $\mu\text{g}$ . or 1 g, of mammalian DNA is needed to elicit an equivalent biological effect.

Conversely, if a single oncogene is present in 1  $\mu\text{g}$  of mammalian DNA, the equivalent amount of the same oncogene if cloned in a plasmid vector is  $1 \times 10^{-6}$   $\mu\text{g}$ , or  $1 \times 10^{-3}$  ng (1 pg).

### Oncogenicity of src DNA in Chickens

#### Fung *et al.* (1993)

- Cloned RSV DNA (2  $\mu\text{g}$ ) induced tumors in 6/6 chickens inoculated s.c. in their wing-web
- Cloned v-src DNA (2  $\mu\text{g}$ ) induced tumors in 7/10 chickens inoculated s.c. in their wing-web

#### Halpernet *et al.* (1990)

- Cloned v-src DNA (20  $\mu\text{g}$ ) induced tumors in chickens
- 52/60 (87%) inoculated s.c. in their wing-web
- 8/36 (22%) inoculated i.v.

#### Conclusion

2  $\mu\text{g}$  ( $2.5 \times 10^{11}$  molecules) of cloned v-src oncogenic in chickens

### Oncogenicity of ras DNA in Mice

#### Burns *et al.* (1991)

- Activated H-ras (T24) gene (10  $\mu\text{g}$ ) inoculated by scarification of mouse skin
- Lymphangiosarcomas developed in 33/34 animals within 12 months; usually within 12 weeks
- Normal c-ras failed to induce tumors (0/10 animals)

#### Conclusion

10  $\mu\text{g}$  ( $1.1 \times 10^{12}$  molecules) of activated ras is oncogenic in adult mice

### Oncogenicity of Polyoma Virus DNA *in vivo*

- Polyoma virus DNA in newborn hamsters
 

i.p.	0.5 $\mu\text{g}$ supercoiled	5/52 (15%)
s.c.	0.5 $\mu\text{g}$ supercoiled	14/73 (19%)
s.c.	0.5 $\mu\text{g}$ linear	29/164 (45%)
- Cloned polyoma virus DNA in newborn hamsters
 

s.c.	0.5 $\mu\text{g}$ supercoiled	11/20 (55%)
s.c.	2 $\mu\text{g}$ supercoiled	0/31 (0%)
s.c.	2 $\mu\text{g}$ linear	33/55 (60%)
s.c.	0.2 $\mu\text{g}$ linear	2/9 (22%)

#### Conclusion

0.2  $\mu\text{g}$  ( $1.9 \times 10^{10}$  molecules) of polyoma virus DNA is oncogenic in newborn hamsters

<b><i>in vivo</i> Infectivity of Cloned Viral Genomes - Retroviruses (1)</b>			
Viral DNA	Dose/Route	Infected/Total Animals	Genomes for Infection
SIV (Haseltine)	200 µg i.m.	3/4	$6.1 \times 10^{12}$
SIV (Ruprecht)	500 µg i.m.	3/3	$3.5 \times 10^{11}$
SIV (Purcell)	300 µg i.m.	2/2	$2.3 \times 10^{13}$
	15 µg i.d.	2/2	$1.1 \times 10^{12}$
BLV (Willems)	100 µg DOTAP i.d.	5/5	$5.5 \times 10^{12}$
	100 µg i.d.	1/1	$5.5 \times 10^{12}$
CAEV (Suzan)	100 µg DOTAP i.a.	5/5	$6.0 \times 10^{12}$
FeLV (Roy-Burman)	50 µg DOTAP i.d.	13/13	$3.0 \times 10^{11}$
FIV (Luciw)	30 µg i.d.	1/3	$6.4 \times 10^{12}$
	30 µg i.m.	2/3	$3.1 \times 10^{12}$
	100 µg i.m.	3/3	$7.0 \times 10^{11}$
	300 µg i.m.	3/3	$2.1 \times 10^{13}$

<b>In vivo Infectivity of Cloned Viral Genomes - Retroviruses (2)</b>					
D	N	A	DNA/Route/Infected/Total Animals	Genomes for Infection	
			Murine FrCas <sup>E</sup> (Portis) 38 µg i.p.	16/16	$2.7 \times 10^{12}$
			0 µg	OH4	
			19 µg i.p.	9/12	$1.8 \times 10^{12}$
			3.8 µg i.p.	7/10	
			0.38 µg i.p.	0/6	$3.9 \times 10^{11}$
			0.038 µg i.p.	0/8	
			FrCasE supercoiled 36 µg i.p.	0/16	$2.7 \times 10^{12}$
			FrCasE linear 38 µg i.p.	13/13	

<b>Summary of <i>in vivo</i> Infectivity with Cloned Viral Genomes</b>		
Viral DNA	DNA/Route	Genomes for Infection
Retroviruses	15 - 500 µg i.m.	$1.1 \times 10^{12}$ - $2.3 \times 10^{13}$
Polyoma Virus	5 x $10^{-5}$ µg - 1 µg s.c.	$1.3 \times 10^7$
<b>Conclusions</b>		
<ul style="list-style-type: none"> <li>Infectivity of different retroviral DNAs is similar</li> <li>Depending on the route of inoculation, 15 µg can be infectious</li> <li>Infectivity of polyoma virus DNA is higher (ca. 50 pg)</li> </ul>		

<b>Comparison of Oncogenicity and infectivity</b>		
DNA	Oncogenicity	Infectivity
Polyoma Virus	0.2 µg ( $3.6 \times 10^{10}$ genomes)	ID, $1.3 \times 10^{-4}$ µg ( $2.3 \times 10^7$ genomes)
SV40	1 µg ( $1.7 \times 10^{11}$ genomes)	ND
Retroviruses	NR ( $1.1 - 2.2 \times 10^{12}$ genomes)	15 - 30 µg
v-src	2 µg ( $2.5 \times 10^{11}$ molecules)	NR
Activated ras	10 µg ( $9.1 \times 10^{11}$ molecules)	NR
ND not done NR not relevant		

<b>Activity of DNA Administered Intranasally</b>		
<ul style="list-style-type: none"> <li>Influenza virus HA gene DNA vaccine inoculated in mice via nasal route</li> <li>Different amounts of DNA were administered in PBS (10 µL)</li> <li>After 4 weeks, a second intranasal inoculation of 1.5 µg was given</li> </ul>		
HA DNA	Immune Response (HI; IgA)	
	Primary Inoculation	Secondary Inoculation
0 µg		
0.02 µg		++
0.4 µg		++
6 µg	+	++
<b>Conclusion</b>		
Even 0.02 µg DNA (i.e., 20 ng) administered i.n. elicits an immune response, since a secondary inoculation boosts the response. Therefore, 20 ng of DNA can be biologically active when administered intranasally.		

<b>Activity of DNA Administered via the Oral Route (1)</b>	
<b>Polyoma Virus DNA</b>	
<ul style="list-style-type: none"> <li>Mice fed polyoma virus DNA (1 µg or 0.5 µg) 0/25 became infected with 1 µg 0/30 became infected with 0.5 µg</li> <li>Mice administered polyoma virus DNA (1 µg or 0.5 µg) through a gastric tube 5/10 became infected with 1 µg 11/18 became infected with 0.5 µg (ID<sub>50</sub>, 0.81 µg)</li> </ul>	
<b>Conclusion</b>	
Unlikely that small amounts of DNA will survive passage through stomach intact	

### Activity of DNA Administered *via* the Oral Route (2)

#### Phage DNA

- Mice fed M13 DNA (50 µg); fate of DNA followed
- DNA detected in: feces (size 100 bp - 1700 bp; M13 DNA 7.25 kb)  
blood (size 194 bp - 976 bp)  
small intestine  
large intestine  
leucocytes (size <712 bp); found in 0.1% cells

#### Conclusions

- Small amounts of DNA can pass through the stomach
- No full-length DNA detected
- DNA can be found integrated into the mouse chromosome

### Estimates of DNA Risk: Oncogenicity

#### Cloned Activated Oncogene

For a single dominant activated oncogene per cell, 1 µg cell DNA has 152,000 oncogenes (DNA in 152,000 cells is 1 µg)

Number of molecules required for oncogenicity:

polyoma virus DNA  $3.6 \times 10^{10}$   
activated *ras* DNA  $9.1 \times 10^{11}$

Therefore, the number of tumors expected from 1 µg of residual cell DNA with a single dominant oncogene per cell is between:

152,000/3.6 x 10<sup>10</sup> = 4.2 x 10<sup>-6</sup>  
and 152,000/9.1 x 10<sup>11</sup> = 1.7 x 10<sup>-7</sup>

Therefore, for 10 ng DNA, the probability of an oncogenic event is between:

1 in 2 x 10<sup>5</sup> (polyoma virus) and 1 in 6 x 10<sup>8</sup> (act. *ras*)

### Estimates of DNA Risk: Infectivity

#### Polyoma Viral DNA

Viral genomes required for infection: 1 x 10<sup>7</sup>

The probability of an infectious event for 10 ng of mammalian DNA with a single copy of polyoma virus DNA is:  
1 in 7 x 10<sup>3</sup>

#### Retrovirus Cloned Proviral DNA

Viral genomes required for infection: 4 x 10<sup>11</sup>

The probability of an infectious event for 10 ng of mammalian DNA with a single provirus is:  
1 in 3 x 10<sup>8</sup>

### Conclusions from Estimates of DNA Risk

• Infectious risk of DNA can be more important than oncogenic risk

• i.m. and s.c. routes

10 ng DNA provides an estimated risk:

Polyoma virus DNA: 1 in 2 x 10<sup>5</sup> for oncogenic event

1 in 7 x 10<sup>3</sup> for infectious event

Activated *ras* DNA: 1 in 6 x 10<sup>6</sup> for oncogenic event

Proviral DNA: 1 in 3 x 10<sup>8</sup> for infectious event

• i.n. route

10 ng DNA provides an estimated safety margin of:

1 in 10<sup>6</sup> for an "expression" event

• Oral route

1 µg polyoma virus DNA administered orally is not infectious; therefore, for 10 ng of mammalian DNA with 1 copy of polyoma virus DNA, the safety margin is at least 1 in 10<sup>8</sup>

### Potential Mitigating Factors Regarding DNA Risks

- Uptake, expression, and integration are inefficient processes
  - integration requires cell division
  - Integration required for maintenance of DNA
- Degradation of DNA
  - Vaccine manufacture procedure
  - *in vivo*
- Host immune response to transfected cells.
- Multistep nature of carcinogenesis
- Transformation of human cells is more difficult than transformation of rodent cells

### CBER Activities to Address the Risks of Residual Cell-Substrate DNA

#### CBER/NCI/CDER DNA Oncogenesis Study

• To develop sensitive animal models to detect oncogenic activity of DNA

- newborn NIH Swiss anh C57Bl6 mice, athymic (nude) mice, and K6/ODC mice

- *myc* and activated *ras* genes

#### CBER DNA Infectivity Study

• To develop quantitative *in vitro* assays to assess:

- the infectivity of proviral DNA of retroviruses  
- the integrated and extrachromosomal genomes of DNA viruses