Insertional Mutagenesis and Autoimmunity Induced Disease Caused by Human Fetal and Retroviral Residual Toxins in Vaccines

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Objectives

- Understand vaccine manufacturing and cell substrate residual contaminant levels.
- Gain knowledge regarding species specific insertional mutagenesis and autoimmunity.
- Understand the relationship of these pathological processes to current childhood disease epidemics including autistic disorder, leukemia, lymphoma, intellectual disability, schizophrenia and bipolar disorder.

Introduction

Major concern of vaccination regarding childhood diseases in terms of Insertional Mutagenesis and Autoimmunity

The potential consequences of injecting our children with human fetal DNA contaminants include two well-established pathologies:

1) Insertional mutagenesis in which fetal DNA incorporates into the child's DNA causing mutations.

2) Autoimmune disease triggered by the human fetal DNA in vaccines leading a child's immune system to attack his or her own body.

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Vaccines

United States manufacturing process and vaccine history of using human fetal cell lines:

- In January 1979, the rubella manufacturing switch from animal based to the human fetal cell line WI-38 was approved by the FDA. A newly approved monovalent rubella vaccine and a trivalent mumps, measles and rubella vaccine both utilize the WI-38 fetal cell line for manufacturing.
- In November 1987 a human fetal cell line manufactured polio vaccine was FDA approved, which was discontinued in the US after 1991.
- In 1989, a second dose of trivalent fetal manufactured vaccine against mumps, measles and rubella was recommended for children at 12 months or older, and a measles vaccination compliance campaign was launched that doubled trivalent fetal manufactured mumps, measles and rubella vaccination rate.
- In 1995, a vaccine against chickenpox that was manufactured using the human fetal cell lines WI-38 and MRC-5 was approved by the FDA.

In general, a vaccine is a vial that contains a virus or a subunit of a virus, a liquid buffer, and contaminants from the cell line that was used to manufacture the virus. Some vaccines also contain preservatives or adjuvants, such as thimerosal or salts of aluminum. The viruses to be used in the vaccines are manufactured in cells or cell lines because they are too large to be made economically through synthetic means. Therefore, the manufacturers take advantage of the natural way that viruses replicate by infecting cells or cell lines with the virus and then harvesting the virus after it has replicated itself thousands or millions of times over.

Concerns regarding human derived cell lines for manufacturing

A cell line originally comes from a live animal or organism (the primary cells), but then genetic modifications are made in many cases to the primary cells such that they become long-lived and can be grown in the laboratory for years and even decades without having to go back and get more primary cells from the animal or organism. When the source that the cell line was ultimately made from was an electively aborted fetus, the manufacturers call these "human diploid cell lines." Currently, if you see these words on the package insert of a vaccine or a drug, or listed in the ingredients of a cosmetic, the cell line was derived from an electively aborted baby. For example, HEK293 was derived from the kidneys of an aborted fetus and immortalization was accomplished by transformation with Ad5 E1A and E1B gene functions (adenoviral DNA). MRC-5 or WI-38 are examples of two fetal cell lines used for vaccine manufacture that were derived from embryonic lung that have not been immortalized and possess only a finite life span (~50 population doublings).[1]

WHO/FDA guidelines and threshold

In early guidance meetings, regulatory agencies and experts initially argued for a recommended limit of 10 pg contaminating cell substrate DNA per dose,[2] which was later on relaxed to 100 pg in 1986 (World Health Organization Study Group; Geneva).[3] After another change based on a WHO meeting in 1997, the currently recommended maximal amount of residual cell-substrate DNA per dose in a vaccine produced in a continuous cell line is 10 ng.[4] Neither limit was based upon empirical study or data to justify the guidance.

Excerpt from the FDA Briefing Document September 19, 2012 (p 25):

Vaccines and Related Biological Products Advisory Committee Meeting

The value of 100 pg of host cell DNA per vaccine dose remained the recommended standard for a decade. However, the issue was revisited in 1997 for several reasons. First, vaccine manufacturers could not always meet this level of residual cell-substrate DNA for some viral vaccines, such as with certain enveloped viruses. Second, more information was available as to the oncogenic events in human cancers, where it has been established that multiple events, both genetic and epigenetic, are required.*[5-9] And third, for continuous non-tumorigenic cell lines such as Vero, the major cell substrate that was being considered at the time, the presence of activated dominant oncogenes in these cells was unlikely. The outcome of the 1997 WHO meeting was that the amount of residual cell-substrate DNA allowed per dose in a vaccine produced in a continuous cell line and one administered by the parenteral route was raised from 100 pg to 10 ng.*[10] (*reference numbering changed for this publication)

Fragmentation of fetal derived DNA

The same FDA Briefing Document (2012) advises (p 17, 18):

The oncogenic and infectious risk of residual DNA in vaccines can be reduced by the implementation of manufacturing steps designed to lower the amount of DNA, decrease the size of the DNA, and/or to reduce the activity of residual DNA by chemical treatment or gamma irradiation. ... Current recommendations are that the level of residual cell-substrate DNA should be ≤10 ng per dose and a median DNA size of 200 bp or lower.

Summary: Although current testing recommendations include evaluation of the oncogenicity of host cell DNA and cell lysates in vivo, *the oncogenic and infectious risk of DNA is primarily addressed by lowering the amount of DNA*, decreasing the size of the DNA (by nuclease digestion), and/or by reducing the activity of the DNA (by chemical treatment or gamma irradiation).

Content of human fetal DNA in several vaccines above WHO/FDA threshold

The only monovalent rubella vaccine available in the U.S. until 2011 (discontinued), was manufactured using the human diploid cell line WI-38 and contaminated with greater than 150 ng cell substrate DNA (sum of dsDNA and ssDNA) per dose, fragmented to approximately 215 base pairs in length. 150 ng of DNA is equivalent to the total amount of DNA in over 22,000 cells. Additionally, this vaccine was contaminated with fragments of the HERVK retrovirus. Another example is a hepatitis A vaccine that is

manufactured using the human diploid cell line MRC-5 and is contaminated with more than 300 ng cell substrate DNA (dsDNA + ssDNA) per vaccine dose.[11] The chickenpox vaccine available in the US is contaminated with greater than 2 µg fetal MRC-5 DNA, according to the manufacturer's measurements.[12]

Recommendations to fragment the contaminating DNA were based on concern that an entire cancer causing gene might be present among the fetal DNA contaminants. However, science has demonstrated that in contrast to the integration of large DNA gene lengths, integration of short DNA fragments has been shown to be much more efficient. Integration is maximal when fragments are between 100 and 1000 base pairs in length.[13-14] Therefore, the recommendations to fragment the contaminating DNA may have increased the danger of the contaminants.

Fetal DNA vaccine contaminants have the potential to cause Insertional Mutagenesis

Mammalian cells can take up extracellular DNA fragments by receptor mediated endocytosis. Uptake is most efficient at low concentrations of extracellular DNA[15] and peaks 2 hours after addition of the DNA fragments to cell culture.[16] In the extracellular concentration range of 0.1 to 7 µM, oligonucleotides (small bits of nucleic acids) readily enter cultured cells through receptor mediated uptake,[17-20] reaching intracellular and nuclear [17, 21-23] concentrations which equal or exceed that of the extracellular medium within 2-4 hours.[24] Empirical experiments have shown that addition of placental DNA fragments of 500 base pairs in length contributed approximately 4% of a cell's genomic content per hour of incubation — roughly 40-50% of fragmented DNA added to cell culture will be taken up by a cell and 10-20% of the added DNA will be delivered to the nucleus, demonstrating the rapidity with which DNA can enter a cell.[15]

Insertional Mutagenesis and Autism Spectrum Disorders

Contaminating fetal DNA fragments might be inserted into a child's genome causing subsequent mutations during the normal process of double strand break repair (DSB). Indeed, it has been demonstrated that genes involved in DSB are differentially expressed in ASD.[25] Faulty DSB is known to be involved in many diseases.[26] DSBs occur both in somatic and germ line cells, and can be programmed, such as in somatic cells for immunoglobulin hypermutation and class switching, or the result of DNA replication, spontaneous DNA hydrolysis or cellular metabolism.[27-28] Toxins and chemotherapeutics can be inducers of DSBs in somatic cells. In the case of various lymphomas, we know that the addition of a toxin or chemotherapeutic induced DSB on top of a programmed class switching DSB leads to cancer.[28] In summary, this research reveals that the genetic susceptibility of some children to the development of ASD is due to the genes involved in DSB being differentially expressed (i.e. not normal). Together with the presence of recombination hotspots in genes that have been associated with ASD, these differentially expressed DSB genes constitute an underlying predisposition to development of ASD as

a result of insertions of fetal DNA. Thus, children with this genetic condition (abnormal DSBs) are extremely susceptible to such insertions.

Meiotic recombination (MR) involves highly regulated pathways of double strand break (DSB) formation and repair. MR occurs at clustered sites within the human genome, termed recombination hotspots, the vast majority of which are located outside of genic regions,[29] presumably to reduce the potential for lethal results after MR. Interestingly, sites of MR/HR (homologous recombination) have been demonstrated to be further susceptible to additional DSBs and mutations.[30-32] Over 350 genes have been associated with autism spectrum disorders. Genomic anomalies include common genetic variations,[33] changes in chromosomal structure,[34] and rare mutations.[35] Recently, *de novo* deletions and duplications have been identified in up to 10% of simplex autism spectrum disorders, indicating environmental influences on the genetics of autism spectrum disorders.[36-37] 10% may well under-represent *de novo* mutations (DNM) as methods are limited to detecting large *de novo* CNVs (copy-number variations) and do not fully capture smaller mutations.[38] Furthermore, each specific mutation is found in only a very small percent of cases, highlighting the complexity of genomic impacts on autism spectrum disorders and the challenge of understanding the *de novo* mutation process. Network mapping is revealing downstream links between these diverse genomic mutations and autism spectrum disorders phenotype,[39] yet we do not understand the process by which diverse genomic sites are targeted for mutation. However, recombination hotspots are concentrated in the genes that have been associated with autism, and may contribute an underlying susceptibility to mutations in those genes when presented with fetal DNA fragments.[11,40] Altered double strand break formation and repair pathways (DSB) may be a commonality among the extremely diverse genetic mutations observed in autism spectrum disorders. Unfortunately, the focus of concerns amongst scientists in academia, in industry and at the FDA has been on the potential of residual DNA for oncogenicity or infectivity, not on the potential for the induction of subsequent gene mutations following genomic insertion of DNA fragments, although this danger was indeed discussed during a 1999 FDA workshop entitled "Evolving Scientific And Regulatory Perspectives On Cell Substrates For Vaccine Development."[41] Numerous studies have established the ability of species specific DNA to accumulate intracellularly and insert into a host's genome at an appreciable rate, especially as DNA fragments in the form of very small chromatin-like particles (natural "DNA nanoparticles").[42-43]

Insertional mutagenesis and other neurodevelopmental disorders

Beside autism spectrum disorders epidemic, there are also apparent epidemic levels of other early onset neurodevelopmental syndromes such as childhood onset schizophrenia (0.4% of population affected),[44] and bipolar disorder.[45-46]

The continued or rising prevalence of these early onset neurodevelopmental diseases despite the reduced reproductive fitness associated with them implies important non-heritable genomic and environmental components to the diseases.[47] Accumulating evidence from family-based exome sequencing approaches published over the

past several years points to the importance of *de novo* mutations in these diseases that include simplex autism disorder and autism spectrum, schizophrenia and intellectual disability.[48-56] Hundreds of rare, *de novo* mutations have been identified in individuals with autism disorder or intellectual disability that are related by involvement in large functional networks of genes.[50,57] In the case of schizophrenia, this network involves the glutamatergic systems, and in the case of autism disorder the network involves genes which are important for the formation and function of synapses. The literature is divergent with regards to whether DNMs are found at a higher rate with disease versus the general population. While the rate of DNMs is not uniformly reported as elevated compared to non-diseased children,[58] the DNMs in these diseases are consistently found in exons or critical coding regions of genes that would lead to premature stop or non-functional proteins.[50,51,59] Other investigators, such as Awadalla, found an excess of DNMs in autism and schizophrenia,[49] and the DNMs identified by Hamdan et al., which disrupted protein function in children with intellectual disability were not present in healthy controls.[60] In contrast to the slight increase in DNMs found in children with neurodevelopmental disease, *de novo* genomic insertions and deletions are significantly increased in childhood onset schizophrenia or autism disorder compared to healthy controls (0% versus 10%).[51,55,61]

Vaccines containing HERVK

Human endogenous retrovirus K (HERVK), a contaminant in some of the chickenpox and measles/mumps/rubella vaccines[62] is a retrovirus that integrated into human germline cells relatively recently in human evolution and is inherited in a Mendelian fashion as an endogenous retrovirus. Such retroviruses are generally inactive. Thus, experts have considered the presence of endogenous retroviruses in the human genome to be innocuous. However recent evidence has shown that HERVK can be reactivated[63-66] or even maintain its activity in present day humans[67] and integrase activity from homologous HERVK sequences has been reported.[64] Active HERVK integrates preferentially in transcription units, in gene-rich regions, and near features associated with active transcription units and associated regulatory regions.[68]

Recent evidence has shown that HERVK transcripts are elevated in the brains of patients with schizophrenia or bipolar disorder[69-70] and in the peripheral blood mononuclear leucocytes of patients with autism spectrum disorders.[71] This retrovirus has also been associated with several autoimmune diseases.[72-74] HERVK is in the same family of retroviruses as the MMLV[75] virus used in a gene therapy trial, in which inappropriate gene insertion led to subsequent additional somatic mutations and cancer in 4 of 9 young boys.[76] The HERVK gene fragment present in vaccines more likely than not codes for the integrase or the envelope protein, thus is active and induces gene insertion[64] or neuroinflammation.[77-78]

Example of Insertional Mutagenesis in patient cases

In an early gene therapy trial, the experts with the FDA's Gene Therapy Division estimated that the risk of retroviral and human DNA fragment induced mutations and cancer was 1 in a trillion. Tragically, when they gave the retroviral and human DNA fragments to boys with SCID disease in a gene therapy trial, 4 out of 9 (44%) of the boys developed leukemia.[76] 44% is a lot higher than the FDA's estimated risk of 1 in a trillion.

Autoimmunity

Cause (fetal human DNA fragments)

Scientists have found that children with autistic disorder have antibodies against human DNA in their blood that non-autistic children do not have. These antibodies may be involved in autoimmune attacks in autistic children.[79-81]

Exposure of a child to fragments of human fetal (primitive) non-self DNA could generate an immune response that would cross-react with the child's own DNA, since the contaminating DNA could have sections of overlap closely similar to the child's own DNA.

Antibody measurements in the serum of autistic versus healthy age and sex matched controls demonstrated significantly higher percent positivity of serum antineuronal antibodies (62.5%) than healthy controls (5%). Moreover, the frequency of the presence of these antibodies was significantly higher in female children with autism (90%) than male autistic children (53.3%; 60 males and 20 females; ages range between 6 and 12 years.)[79]

Increased by number of administered vaccines with fetal DNA contaminants and frequency of injections thereof

With the current ACIP and statewide vaccination recommendations, children may be exposed to as many as 7 or more fetal DNA contaminated vaccines before they are 2-3 years old, compared to only 2 vaccines containing fetal DNA in the early 1990s. During the period from birth out to three or more years, human brain development is an active process, with neural circuits being established, pruning of unused dendritic synapses going on, and nerve cell death occurring on a massive scale.[82-83] During periods of intense brain cell death such as this, DNA not otherwise found extracellularly would be present and serve as the target for autoimmune attacks, originally triggered by exposure of a young child to the fetal DNA fragments found in vaccines.

Summary

1. Contaminating DNA levels in the rubella, mumps-measles-rubella, chickenpox and some hepatitis A vaccines available in the US well exceed the current World Health Organization guidance of less than 10 ng cell substrate DNA per vaccine dose.
2. The DNA of the aforementioned rubella vaccine was fragmented into short pieces of approximately 215 base pairs (in average) in length, a length ideal for cellular uptake and genomic integration.

3. Some of the chicken pox and measles/mumps/rubella vaccines are also contaminated with fragments of the Human Endogenous Retrovirus K (HERVK), a retrovirus that invades the genome of its host, can be re-activatable and which can facilitate the integration of stray DNA into the host's genome.
4. Short DNA fragments are known to integrate into the genome in a species specific manner and can lead to mutagenesis and/or genomic instability as well as an autoimmune response.
5. The vaccine schedule exposes young children to insertion of fetal DNA fragments during a time of significant brain development.

The dangers of retroviral fragments as well as residual human diploid DNA are an unstudied risk to vaccine recipients, and yet, the overwhelming body of scientific literature clearly demonstrates the high likelihood of autoimmune and/or insertional mutagenesis dangers from these contaminants. This is an issue that undoubtedly cries out for serious epidemiological and scientific investigation. SCPI is currently conducting a study to provide further clinical proof for autoimmunity caused by fetal DNA found in vaccines (see Addendum at the end of this publication for additional details).

Recommendations

- *Disclosure* of fetal DNA quantities in vaccine package insert.
- *Alternatives* that are already available and manufactured in other countries.

Vaccines can be safely and effectively manufactured in animal, insect or plant based cell lines, eliminating the dangers of residual human DNA and retroviral contaminants:

A rubella vaccine available in Japan, which is based on Takahashi strains of live attenuated rubella virus, *is produced on rabbit kidney cells*. A single dose of this vaccine has been recently proven to retain immunity for at least 10 years when rubella was under regional control.[84]

Addendum
Further clinical proof or currently conducted studies for autoimmunity and insertional mutagenesis caused by human fetal DNA found in vaccines

Autoimmunity

During the past several years multiple scientific publications have demonstrated that approximately 40% of children with simplex autism have immune responses to neural tissue and more importantly, to human DNA that typically developing children do not have.[79-80, 85-87]

SCPI is currently conducting an Institutional Review Board approved observational clinical trial, in collaboration with Dr. Karin Burkhard, M.D., to determine whether children with autism also have immune responses to the specific human fetal contaminants found in the suspected childhood vaccines. Dr. Burkhard is a psychiatrist in Hauppauge, NY, who received her medical degree from the Geisel School of Medicine at Dartmouth and has been in clinical practice for more than 20 years. We have already enrolled 20

autistic and 20 typically developing children for this study, which will determine the immunity for each individual child to the following:

- general human DNA,
- the specific human fetal DNA from cell lines MRC-5 and WI-38, and
- their own DNA (autoimmunity)

Evidence for immunity to the human fetal DNA contaminants as well as autoimmunity to the children's own DNA will provide compelling proof for the dangers of using human fetal cell lines to manufacture vaccines due to the reasons presented in this publication.

Insertional Mutagenesis

It seems reasonable that the fetal contaminants in vaccines could cause a disease like cancer, because cancers are known to start due to a mutation in just one cell, but how could a mutation in one cell cause a diffuse neurodevelopmental disorder like autism?

Cancers such as lymphoma and leukemia are known to be clonal. Clonal means that all of the cancer cells arise from a single mutated cell. Typically, the originating cell will have a mutation that gives it a survival advantage over other cells. While it makes sense that a single cell could take up the human fetal DNA contaminants found in vaccines, undergo insertional mutagenesis and lead to cancer, it seems less obvious how a single cell could lead to a diffuse neurodevelopmental disease like autism. Well, the field of hematology has shown that our blood system is largely clonal.[88-89] We have trillions of blood cells in our bodies, however, it turns out that just a very few blood stem cells are active and make all those trillions of blood cells.

How could a mutation in a hematopoietic stem cell (HSC) cause problems in the brain? Glial cells found in our brains are generated from the differentiation of HSCs in our bodies. HSCs circulate periodically and then return to the bone marrow. While circulating, one of these stem cells could readily take up human fetal DNA fragments causing insertion into the cell's DNA and a mutation, as small fragment homologous recombination has taught us, readily happens in blood forming stem cells.

What this means is that while we have millions of stem cells, in most people only 7 or 8 stem cells are actively making all the trillions of blood cells in our bodies. In many people, only 1 or 2 stem cells make up to 90% of the trillions of blood cells in our bodies, which means a mutation in a single blood stem cell, which typically gives the mutated cell a survival advantage as seen with cancer, could result in 50% or more of our blood cells carrying the same mutation. Furthermore, the glial cells that populate our brains can be replaced during life with new glial cells from the blood. If those replacing glial cells are formed by a mutated blood stem cell, then the glial cells in the brain could carry a dominant mutation. Mutated glial cells in the brain could cause a diffuse abnormal immune activity in the brain, and glial cells are also known to be critically important for nerve cell signaling.

Thus, a mutation in a single blood stem cell is quite probable when children receive human fetal DNA contaminants in their vaccines. Such a mutation would give

that cell a survival advantage, and that mutated cell could produce trillions of mutated blood cells that would subsequently populate the brain's glial compartment and lead to diffuse abnormal brain function in these children. This mechanism appears to be the cause of simplex autism in about 60% of children, while the other 40% appear to have an autoimmune mediated regressive autism.

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