

# **Respondent's Exhibit V**

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### **Qualifications**

This report is based on my knowledge of the literature and on the research that I have conducted on measles and the immune responses to measles virus over more than 20 years. I have reviewed the laboratory data, expert reports and relevant medical records for this case.

My CV and bibliography are attached. As therein documented, I have trained in the research disciplines of both immunology and virology and have clinical training in infectious diseases. I have conducted research on the pathogenesis of a number of viral diseases and, in particular, the outcome of infection with wild type and vaccine measles viruses. I have specialized in studies that aim to characterize the interactions of measles virus (MV) with the immune system. These studies have included investigations of children with measles in both developed and developing countries, and responses of adults and children to immunization with measles vaccine and measles-mumps-rubella (MMR) vaccine in developed and developing countries. These studies have been published in critically reviewed, highly regarded and frequently cited journals. I have written the chapters on measles in reference textbooks such as *Field's Virology*, *Infections of the Central Nervous System* and *Encyclopedia of Molecular Medicine*. In addition, I have also conducted research on viral encephalitis and mechanisms of viral clearance from neurons.

I am the president of the American Society for Microbiology, past president of the American Society for Virology and of the Association of Medical School Microbiology and Immunology Chairs and Retiring Chair of the Medical Sciences Section of the American Association for Advancement of Science (AAAS). I was an Editor for the *Journal of Virology* for 10 years and am on the editorial boards of *Virology*, *Journal of Neurovirology*, *Virus Research*, *Journal of Clinical Investigation* and the *Proceedings of the National Academy of Sciences*. I serve on the Scientific Advisory Council for the Alberta Heritage Foundation for Medical Research. I am a fellow of the Infectious Diseases Society of America, of the AAAS and of the American Academy for Microbiology. I am a member of the National Academy of Sciences and the Institute of Medicine.

My primary areas of expertise related to these pleadings are in the areas of immunology and virology.

### **Measles**

Measles is one of the most infectious of viral diseases. The natural wild-type measles virus (MV) infection is spread from one individual to another by the respiratory route (aerosols) and is a significant cause of morbidity and mortality worldwide. MV enters the airways and infects epithelial cells lining the respiratory tract. From there, the virus spreads to local draining lymph nodes either through infection of dendritic cells or monocyte/macrophages resident in the lung. These cells are capable of taking up and transporting infectious organisms to lymphoid tissue where the immune response is initiated. In the lymph nodes the virus replicates in macrophages and lymphocytes and probably in dendritic cells (Moench et al, 1988; McChesney et al., 1997).

From the lymph nodes the virus spreads through the blood (Esolen et al, 1993), to many organs including the skin, liver, kidney, gastrointestinal tract, conjunctivae, lungs, spleen and other lymphoid tissues including the thymus and appendix. In these target organs the virus replicates in endothelial cells lining the blood vessels, in epithelial cells and in monocyte/macrophages (Moench et al, 1988; McChesney et al., 1997). In children who have died of measles, endothelial cells in the brain may have been infected, but other brain cells are not so infected, and no associated pathology has been recognized (Esolen et al, 1995). Michelle Cedillo had normal brain CT and MRI scans and therefore, no indication of infection of the brain. Virus can be routinely isolated from blood during the first 2 weeks after infection (van Binnendijk et al, 1994) and can be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) for 1 to 5 months after the onset of rash (Permar et al, 2001; Pan et al, 2005; Riddell et al, in press). At peak viremia 1/100-1/1000 of all circulating white blood cells are infected with MV (van Binnendijk et al, 1994, Auwaerter et al, 1999). If each infected cell has 1000 copies of viral RNA, this peak level of infection would result in  $10^2$ - $10^3$  copies/ng RNA (assuming 100 cells have a total of 1 ng RNA).

MV can involve the gut lymphoid tissue, such as the appendix, and epithelial cells of the stomach and small intestine (Moench et al, 1988), but does not usually cause gastrointestinal symptoms (e.g. diarrhea or constipation). Diarrhea can complicate measles, but it is most often caused by secondary infections with gastrointestinal pathogens (e.g. bacteria like *Salmonella* or *Campylobacter*), not MV. This increased susceptibility to other infections is characteristic and accounts for much of the morbidity and mortality due to measles. The mechanism of measles-induced immunosuppression is not well understood, but has its onset during the immune response to MV infection (see below).

MV is a negative sense (or minus-strand) RNA virus that is in the paramyxovirus family. The "sense" of the genome is determined in reference to "message-sense" for messenger (m)RNA, the sense needed for translation of proteins. In contrast to positive-sense RNA viruses, the first step in replication of negative-sense RNA viruses is synthesis of positive-sense mRNA for translation (synthesis) of viral proteins. Related viruses that infect humans include respiratory syncytial virus, mumps virus and parainfluenza viruses. Replication of these viruses takes place entirely in the cytoplasm. Because replication does not have a DNA intermediate and does not require the nucleus, the viral genome

cannot integrate into the host DNA and does not lead to traditional latency (as it can with DNA viruses and retroviruses, e.g. HIV).

MV is restricted to primates (does not naturally infect or cause disease in other animals) and natural wild-type strains of MV are highly adapted for causing disease in humans. The process of growing the virus in nonhuman cells selects for MV variants with a decreased ability to cause disease (Takeda et al, 1998; Takeuchi et al, 2000). The first MV to be grown in tissue culture was the wild type Edmonston virus that was isolated from the blood of a child with measles (Enders and Peebles, 1954). This human virus was attenuated (made less virulent/less likely to cause disease) for development of a vaccine by adaptation for growth in nonhuman cells, specifically chicken cells (Katz et al, 1958). During adaptation, wild type MV lost the ability to replicate efficiently in humans and monkeys, but still replicated well enough to induce immunity and protect monkeys from experimental infection with wild type MV and children from acquiring measles when exposed to MV in the community (Enders et al, 1962; Katz et al, 1959, 1960). Further passages in chick cells led to more attenuation and the vaccine strains in current use today (Schwarz, 1962). These vaccines have proven safe and effective and have saved the lives of many millions of children (CDC, 2002).

Because the vaccine virus has been adapted to growth in chicken cells, and thus has lost properties essential for efficient replication in humans, it replicates much less well in humans than the wild type virus. At times of peak viremia amounts of virus in the blood are small (about 1000-fold less than wild type virus) and replication in other tissues is limited in amount (van Binnendijk et al, 1994). Virus is occasionally detected in urine or the respiratory tract, most often when there is fever or rash, consistent with a low-level viremia (Jenkin et al, 1999; Morfin et al, 2002; van Binnendijk et al 1994). Fever or rash occurs in about 10% of immunized infants 5-10 days after vaccination, and Michelle Cedillo's fever 1 week after MMR may indicate that she experienced this reaction. As judged by the rash complication, the replication and spread of the measles vaccine virus is not detectably different when it is combined with the mumps and rubella vaccine viruses in MMR (Minekawa et al, 1974; Stokes et al, 1971) than when it is used alone.

Because the attenuated measles vaccine virus replicates less well and causes fewer acute symptoms than infection with the wild-type MV, the complications associated with vaccine virus infection are expected to be substantially less severe and less frequent than the complications of natural infection with wild-type virus. Specifically, one would not expect to observe complications with the vaccine that have not been observed with natural infection.

### **Immune response to measles virus**

There are 3 well-recognized phases of the immune response to a systemic virus infection such as measles: (1) induction of the immune response to the virus, (2) proliferation and distribution of immune effector cells and their products (antibodies, cytokines) to the sites of virus infection resulting in control or clearance of the virus, and (3) elimination of immune effector cells that are no longer needed after the virus is cleared and immunologic memory has been established.

During the *first phase*, the virus is replicating and spreading to new cells. Initial interaction of MV with the immune system probably occurs in the respiratory tract where tissue-based immature epithelial dendritic cells take up the virus and transport it to the local lymph nodes for initiation of the immune response. As these dendritic cells move from the respiratory mucosa to the lymph node they undergo a maturation process to be better able to stimulate naïve lymphocytes (T cells and B cells) in the lymph nodes and to become activated, proliferate and differentiate. During the latter stages of this first phase of the immune response, the host becomes febrile, probably in response to the early production by macrophages, dendritic cells and other infected cells of “proinflammatory” cytokines that influence the responses of virus-specific T cells.

During the *second phase*, naïve CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and B cells that are in the lymph nodes, where dendritic cells with virus have gone, and have the correct surface receptors to recognize the viral antigens, are stimulated to proliferate (so there are more of them), and to differentiate into effector cells that can control and eliminate the virus and virus-infected cells. This is a complicated biologic process that involves multiple cells interacting with each other, expression of a variety of cell surface molecules that promote cell-cell interactions and production of soluble factors that support cell growth and differentiation. B cells differentiate into plasma cells that make antibody to MV. These cells first make IgM and then switch to IgG. These antibodies can neutralize virus that is being produced so more cells don't get infected. Cytotoxic T cells can eliminate virus-infected cells and the cytokines they produce can inhibit virus replication in cells that are not or cannot be eliminated.

Differentiated and activated virus-specific T and B cells leave the lymph node, enter the blood stream and go to the many sites in the body where MV is replicating including skin epithelial cells. This is the time that the rash appears due to infiltration of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes and monocytes into sites of virus replication in skin (Polack et al, 1999). A similar phenomenon is presumably occurring at all other sites of virus replication, but cannot be visualized so easily.

Virus that is released from infected cells (and therefore available to spread infection to new cells) is quickly prevented from infecting new cells by the presence of neutralizing antibody produced by mature B cells. At this point virus is no longer able to spread, except inside cells. Elimination of MV-infected cells is presumed to occur by CD8<sup>+</sup> T cell-mediated killing of these cells (Permar et al, 2003; Jaye et al 1998; van Binnendijk et al, 1990). Most of the cells that MV infects (epithelial cells, lymphocytes, monocytes and endothelial cells) (Moench et al, 1988; McChesney et al. 1997; Esolen et al. 1993, 1995) are replaced regularly. In addition, MV is relatively easily inactivated (i.e. infectivity is lost) by exposure to warm (i.e. body) temperatures.

Michelle Cedillo had a normal immune response to the MV and rubella components of MMR as evidenced by the production of virus-specific antibodies. The value of 2.96 for MV-specific IgG demonstrates successful vaccination against measles and the lack of MV-specific IgM indicates that infection was in the past. Dr. Kinsbourne states that

“mercury-induced immune dysfunction allowed a live attenuated vaccine virus to persist in her body”. However, the response to this vaccine, as well as other vaccines (e.g. polio, diphtheria, tetanus) was normal. The MV-specific antibody induced in response to MMR will neutralize any new MV produced by vaccine virus-infected cells so that the virus cannot spread from these cells to new cells. The assertion by Dr. Byers that virus coming from the gut can attach to monocytes in the blood and continue to infect the brain is a rather fanciful assertion that is in contrast to the principle that virus released from cells does not continue to infect new cells after production of neutralizing antibody. No vaccine strain MV has been isolated from any site in Michelle and no evidence for persistence of MV capable of continued replication or infection is provided.

During the *third phase* two things need to be accomplished: (1) adjusting the numbers of immune cells back to the pre-infection state and (2) establishing immunologic memory to prevent reinfection. During the effector (second) phase the number of lymphocytes has been greatly expanded. Because the lymphocyte compartment of the body has a finite capacity (we cannot infinitely expand the total number of lymphocytes that we each have), the numbers of virus-specific lymphocytes need to be decreased to allow for the same process of expansion to occur when the host is infected with a different virus or other micro-organism (bacteria, parasite, etc) in the future. Lymphocytes and macrophages that have been activated to deal with the virus need to be “deactivated”. This process is not completely understood, but involves death of activated cells, evolution of subsets of regulatory T cells, and production of “down-regulatory” cytokines like IL-10 and TGF- $\beta$ . During this process, some of the deactivated cells survive and become long term memory T cells and B cells that provide protection from reinfection.

Michelle Cedillo successfully established a memory response to MV as evidenced by the presence of antibodies 2 years after vaccination. The report of Dr. Byers refers to “immune dysregulation characterized by a skewed Th1:Th2 ratio and an elevated helper suppressor ratio”. However, as documented in Dr. McCusker’s report and as reported originally by Dr. Gupta, Michelle Cedillo had an immune system that, at the time of testing, was normal for her age.

### **Persistent infection and detection of MV in tissues and cells**

The concepts and definitions of virus persistence and virus clearance are difficult and were mostly formulated prior to modern molecular techniques and current understanding of virus replication and antiviral immune responses. The early definition was simple: can the virus be demonstrated to be present by culturing virus from the tissues or cells of interest? If so, then the virus was replicating and if replication continued for a long period of time, the virus infection was persistent. If not, the virus had been cleared and the individual had recovered from infection. Therefore, in truly persistent infection, infectious virus is continuously produced and the viral proteins and RNA are abundant and easy to detect. These viruses (e.g. hepatitis C virus) do not kill most of the cells they replicate in and the virus undergoes constant mutation of the RNA that leads to continuous production of new versions of the viral proteins that the immune system may not recognize.

Current widely employed techniques involve detecting viral proteins, genomes or mRNAs without detecting infectious virus. These techniques can include staining tissues with antibody specific for viral proteins (immunocytochemistry) or detection of viral nucleic acid (*in situ* hybridization). Most commonly, the very sensitive method of polymerase chain reaction (PCR) is used to detect viral nucleic acid in extracts of tissues or cells. To detect RNA viruses, such as MV, the RNA is first extracted from the cell or tissue and then the reverse transcriptase (RT) enzyme is used to copy the RNA into DNA. Small amounts of this copy (c) DNA can be amplified using specific primers and a second polymerase enzyme that copies the RT-synthesized cDNA by PCR into a large number of copies of DNA. The amplified DNA is then detected by its size on a gel or by the ability to hybridize with a specific probe for the gene of interest. Because of the tremendous amplification in this chain reaction, poorly designed primers or contamination of the sample with small amounts of either RNA or DNA in the laboratory are common problems leading to false positive results. Interpretation of the results of RT-PCR assays requires documentation of rigorously controlled laboratory methods to provide assurance of specificity and reproducibility (see below).

One way that RNA from a virus may persist is by failing to kill a long-lived cell, such as a neuron or heart muscle cell, which was infected (Levine and Griffin, 1992; Tyor et al, 1992; Destombes et al, 1997; Brewer et al, 2001). Different types of long-lived cells vary in their susceptibility to virus-induced cell death and to immune-mediated killing. The RNA seems to be dormant in these cells and can be detected by RT-PCR. Viral proteins are no longer being made in detectable amounts and the animals show no signs of disease. For this type of persistence the virus need not be replicating, will not spread, is not damaging to the infected cell and the infected cell may not be recognized by the immune system. This mechanism of viral RNA persistence will not be applicable to short-lived cells, such as monocytes, neutrophils, many lymphocytes, gut epithelial cells or inflammatory cells in tissue – typical sites of MV infection. The finding of MV RNA, if true, does not imply presence of MV protein, infectious virus or disease.

To eliminate all viral nucleic acid from an infected individual probably requires elimination of all infected cells, all cells that may have engulfed (cleared) these dead cells and all follicular dendritic cells that interacted with the virus during generation of the immune response. Use of RT-PCR has raised the question of whether any virus is ever completely cleared if you look hard enough. It also challenges common definitions of the term “infection”. Usually infection is taken to mean some disease-inducing process rather than mere residual presence of an infectious organism or its genetic material. The important questions are whether viral RNA detectable in tissue is causing any cellular dysfunction, otherwise it is of no clinical significance.

Based primarily on evidence from RT-PCR, performed on RNA extracted from ileal tissue, Dr. Byers stated that Michelle Cedillo had “impaired clearance of measles vaccine from the body” which “indicates that it is found in multiple other organs in the body”. Dr. O’Leary’s laboratory reported receiving blood, in addition to ileum, but there is no report on whether MV RNA was detected in that specimen. No replicating virus has been

isolated from Michelle Cedillo and no tissue changes characteristic of MV infection (giant cells, syncytia, inclusion bodies; see below) (Bullowa et al, 1936; Breitfeld et al, 1973) were observed in any region of the gastrointestinal tract (multiple pathology reports). Therefore, the argument that persistent measles vaccine virus is the cause of her autism and inflammatory bowel disease rests entirely on one RT-PCR result from Dr. O'Leary's laboratory. Assessing the presence, absence or significance of a virus in tissue solely by RT-PCR amplification of viral RNA is problematic for the following reasons.

1. The PCR technique provides no information on whether the viral RNA, if detected, is from a cell that has virus that is replicating; a long-lived cell that has residual RNA that is not replicating, but is protected from degradation; or a long-lived cell that was previously infected and still contains degraded fragments of viral RNA. The location of the viral RNA and its replicative capacity is important to determine which cells may be infected and the relevance of the presence of the viral RNA to the disease that it is purported to cause.

2. The PCR technique provides no information on whether viral proteins are or are not being made. Production of viral proteins is necessary for any immune response to the virus (e.g. inflammation, cytokine production, etc) because proteins or pieces of proteins are the parts of viruses that are recognized by antibodies and T cells. Viral protein production is also necessary for virus to replicate and for new virions to be made. Without this, there can be no virus replication and no spread of virus within the host to other cells or other tissues.

3. The PCR technique is prone to problems of contamination and interpretation. To assure that the results reported are valid requires that samples be assessed "blinded" or "under code" and with a full array of proper controls and standard curves covering the range of amounts of viral RNA deduced to be present. These problems with PCR are well known and standard methods are used in clinical and research laboratories to assure that the data obtained can be interpreted. Problems arising from poor laboratory quality control are numerous. As confirmation of the results of RT-PCR, the DNA PCR product needs to be sequenced to assure that what is being amplified is MV and to determine whether it is a vaccine strain, a laboratory strain or a wild type virus. This information is essential to show that the PCR reactions carried out in Dr. O'Leary's laboratory are not amplifying nonspecific or contaminating DNA or RNA. Sequencing the PCR product is often the only way to detect problems with laboratory procedures, particularly contamination. It is also the only way to prove that the viral RNA is from the vaccine virus, not from wild type virus.

An example of a disease in which viruses are found if you look hard enough, but there is still no evidence that the viruses found are involved in the disease, is multiple sclerosis. To date, at least 20 different bacteria and viruses, including MV, have been reported by one research group or another to cause multiple sclerosis, often after detection of viral genomes by PCR or RT-PCR. However, no virus has yet been accepted as the cause of the disease (Johnson, 1994).

One example of how the literature can be confused by poor laboratory procedures was the report in the very prestigious peer-reviewed journal *Science* of human T lymphotropic virus (HTLV) as the cause of multiple sclerosis (Reddy et al, 1989). In this paper HTLV was found by PCR in PBMCs of 6/6 multiple sclerosis patients and only 1/20 controls. However, these findings were not confirmed by other laboratories (Bangham et al, 1989; Richardson et al, 1989; Fugger et al, 1990; Ehrlich et al, 1991). It is clear from the original publication that the samples studied were not blinded (i.e. were assayed in a way that the investigators knew whether the sample being assayed came from a patient or a control) and that the investigators ran the RT-PCR on the controls only once and ran the assay on the patients multiple times. The authors state in the paper that *"in two out of four experiments (with the patient samples), we could clearly see an amplified band that could be hybridized to the envelope-specific probes, whereas in the other two experiments such clear amplification could not be detected ..."* Any negative for controls was accepted as the result and any positive for patients was accepted as the result (even if other assay runs on the same patient sample had given the opposite result). Most of these points apply to the way in which the study by Uhlmann et al was carried out. Sequencing the PCR product can often reveal these problems, because the sequence will often be the same as the laboratory control RNA or DNA that indicates contamination or may not be MV at all. In the HTLV-1 multiple sclerosis study sequences from all the patients were essentially identical to a positive laboratory control (Reddy et al, 1989), while sequences from patients actually infected with HTLV-1 showed variation from one individual to another (Bangham et al, 1989) strongly suggesting contamination of the PCR reactions in the Reddy laboratory.

The type of quality control problem illustrated above is directly relevant to Michelle Cedillo's case. Laboratory quality control is an important factor in determining the validity of the RT-PCR data generated by that laboratory. A recent study compared the ability of several research laboratories to reliably detect MV RNA in coded samples by RT-PCR (Afzal et al, 2003). Dr O'Leary's laboratory declined to participate in this objective evaluation of laboratory quality. Multiple investigators have now attempted, and been unable, to confirm the finding of MV RNA in individuals with autism (Afzal et al., 2006; D'Souza et al., 2006). Furthermore, the primers reported in the 2002 Uhlmann paper, when used by others, give rise to a large number of false-positive reactions (D'Souza et al., 2006). The basis for the statement by Dr. Krigsman that the assay used is reliable "as an accurate predictor of the presence of measles virus RNA" is an abstract on which he is an author reporting "preliminary results". I can find no evidence that this study was published in the peer-reviewed literature.

As previously described, at peak level of wild-type MV infection,  $10^2$ - $10^3$  copies/ng RNA could be detected by RT-PCR. The ileal biopsy tissue collected on January 31, 2002, from Michelle Cedillo was reported by Dr. O'Leary's laboratory to contain  $1.67 \times 10^3$  copies of MV RNA/ng RNA. If the data provided by Dr. O'Leary are correct, and if it is assumed to reflect the vaccine virus, then this is a suspiciously high number. Considering that the vaccine virus replicates less efficiently than the wild type MV, this number would predict that all of the cells in this section of the ileum were infected and actively producing viral RNA. With such an overwhelming infection it should be easy to detect

virus by other means (immunocytochemistry, *in situ* hybridization, typical measles pathology, etc), but apparently these were not done and none of the pathology readings on the multiple biopsies taken were consistent with active MV infection. This amount of MV vaccine RNA is not biologically plausible and the inconsistencies of these data call into question the validity of the RT-PCR result.

### **Known consequences of MV persistence**

Persistent virus infection of cells in which MV is replicating and causing disease is easily demonstrable in tissues by a number of techniques that are less sensitive than RT-PCR. These include isolation of the virus, immunocytochemical staining of the tissue for MV proteins and routine pathology showing giant cells or inclusion bodies characteristic of MV infection. These types of tests show the presence of MV in immunologically normal individuals with subacute sclerosing panencephalitis (SSPE)(*e.g.* Brown et al, 1989; Herndon and Rubinstein, 1968) due to persistent infection of the CNS with wild type MV and in severely immunologically compromised individuals (*e.g.* HIV infection, congenital immunodeficiency, cancer chemotherapy) with progressive vaccine- or wild type virus-induced pneumonia or encephalopathy (Bitmun et al, 1999; Budka et al, 1996; CDC, 1996; Enders et al 1959; Monafu et al, 1994); all circumstances in which MV causes the diseases observed. It is expected that these tests would be positive in gut tissue from Michelle Cedillo if persistent MV were actively replicating and causing disease.

**SSPE** - The only disease caused by persistent MV infection in immunologically competent individuals is SSPE, a progressive neurological disease of children and young adults. In SSPE the history of measles at the time of acute infection is often described as mild and the child appears normal for an average of 7-10 years before deterioration of intellectual function is apparent. The virus infects long-lived cells in the brain and continues to produce viral proteins translated from viral mRNAs. These proteins and the viral RNAs encoding these proteins are detectably different from those of the original infecting MV (Cattaneo et al, 1989). The mutated proteins are changed in a way that facilitates continued virus spread within the brain and makes the infected cell less visible to the immune system (Cattaneo et al, 1989; Patterson et al, 2001). This persistent infection produces tissue damage, an exaggerated antibody response and progressive neurological disease. SSPE typically presents with mental deterioration, as evidenced by decreased school performance, accompanied by alterations in personality. This is followed by the appearance of movement abnormalities (myoclonus), characteristic electroencephalographic (EEG) changes and progressive neurological deterioration. Death usually occurs within months to years after onset of the disease (Freeman, 1969). There is no evidence that Michelle Cedillo has a progressive neurological disease.

At the onset of neurological disease there is abundant evidence of MV in the brain. Routine pathology demonstrates aggregates of viral proteins (inclusion bodies) that can be seen inside infected cells without using specialized techniques. Examination of these inclusion bodies by electron microscopy first suggested that MV caused SSPE, because the viral structures looked like those of MV (Herndon and Rubinstein, 1968). This led

investigators to stain brain sections for MV proteins and these proteins were specifically and easily detected by immunocytochemical staining. Investigators also found high levels of antibody to MV in the cerebrospinal fluid (CSF) and blood. Subsequently, MV RNA was specifically and easily detected in the brain by *in situ* hybridization and RT-PCR (Godec et al, 1990) and has been detected in CSF (Kawashima et al, 1996; Nakayama et al, 1995; Suga et al, 1996). This disease is accepted to be caused by wild-type MV because there is a characteristic clinical picture accompanied by an elevation of antibody to MV, characteristic abnormal brain imaging (e.g. ventricular dilatation and loss of parenchyma) and EEG (e.g. burst-suppression), and MV is found in the brain by all of the above techniques (*i.e.* there is no difficulty in finding the virus by any of a number of methods) and by many investigators. Inclusion bodies are now recognized to be a characteristic pathologic feature of persistent MV infection of the brain. Michelle Cedillo's EEG showed generalized slowing and her brain CT and MRI scans were normal.

**Measles inclusion body encephalitis (MIBE)** - In individuals who are profoundly immunocompromised due to genetic defects in the immune system, malignancy, chemotherapy or HIV infection, MV can also persist and cause fatal disease associated with giant cell pneumonia or measles inclusion body encephalitis (Enders et al, 1959; Budka et al, 1996). Because of their immune-deficient states, these individuals often do not manifest a rash at the time of MV infection and develop progressive respiratory and/or neurological symptoms beginning weeks to months after infection. These diseases induced by persistent MV infection are usually progressive and fatal and, as with SSPE, virus continues to replicate and is easily and reproducibly demonstrated by a wide variety of techniques (*e.g.* immunocytochemical staining for proteins, *in situ* hybridization and RT-PCR for RNA, and routine histology for giant cells or inclusion bodies) in the lung and/or brain. Giant cells are typical of acute and persistent MV infection of lung and lymphoid tissue while inclusion bodies are typical of persistent MV infection of brain. Susceptibility to progressive measles is associated with abnormalities in cellular immune function, typically manifested by deficiencies in the numbers of T lymphocytes and increased susceptibility to other infections. There is no evidence that Michelle Cedillo has a progressive neurological disease or compromise of her immune response to infection.

**Progressive vaccine-induced disease** - The attenuated measles vaccine virus can also cause progressive fatal respiratory or neurological disease in severely immunocompromised individuals (*e.g.* HIV infection, congenital immunodeficiency, cancer chemotherapy, etc.) months after immunization. The causal relationship between the virus and the disease is accepted because of the history of immunization, evidence of tissue damage that is typical of that caused by MV infection (inclusion bodies and/or giant cells), visualization of MV by electron microscopy, the frequent ability to isolate replicating virus from the affected tissue and confirmation of these findings by independent laboratories (Bitnun et al, 1999; Monafó et al, 1994; Mawhinney et al, 1971; CDC, 1996).

The ease of detection of MV in cases of MIBE, SSPE and vaccine-induced progressive disease means that there are large numbers of copies of RNA (although probably not as much as reported by Dr. O'Leary's lab here), abundant amounts of viral protein being made and ongoing replication of the virus leading to disease. Michelle Cedillo has no evidence of progressive neurological disease and no evidence of giant cells or other tissue abnormalities characteristic of wild type or vaccine MV-induced disease in the tissue reported to be positive for MV RNA. If the virus is replicating in the CNS, increased levels of immunoglobulin and virus-specific antibody will be found in the CSF as well as blood (Vandvik and Norrby, 1973). Michelle Cedillo does not show a pattern of altered production of measles serum antibody that would suggest persistent infection. CSF has not been analyzed.

Therefore, arguments that the measles vaccine virus is causing autism and inflammatory bowel disease in Michelle Cedillo rely entirely on the reported RT-PCR detection of MV RNA in a single ileal specimen.

### **Evidence that MMR is related to autism**

Because MMR is usually given between 12 and 18 months of age and this is a typical age for symptoms of autism to become manifest, causality cannot be inferred from the coincidence of the two events. Because most children receive MMR, this reasoning would blame anything that happened to any child during the next year on MMR.

The best way to identify such causal relationships is with epidemiologic studies that analyze data from a large number of individuals that differ in vaccine histories and developmental disorders. Extensive epidemiologic studies have shown no association of autism spectrum disorders with the MMR vaccine (e.g. Madsen et al, 2002; Honda et al., 2005; Fombonne et al, 2007; Uchiyama et al., 2007).

Much of the evidence that has been used to implicate the MV component of MMR as a cause of enterocolitis and autism is based on studies published by Andrew Wakefield and his collaborators (e.g. Wakefield, 1998; Uhlmann, 2002) in the UK. These reports and their interpretations have come under increasing scrutiny and criticism in the scientific literature. The reliability of the data to support measles vaccine virus persistence is questionable and the claims of persistence require independent objective verification. Even if the vaccine virus persists there is no evidence that it is being recognized by the immune system or that there is virus-induced tissue damage. As explained in the sections on SSPE, MIBE and progressive pneumonitis in immunocompromised children, persistent MV infection results in the formation of inclusion bodies and/or giant cells in affected tissues. The envisioned pathogenesis of how the measles vaccine caused autism is based on the premise that measles vaccine virus persists, is replicating and causing disease in the gut and the brain. There is no biologic plausibility to these proposed mechanisms based on current knowledge of MV biology and immune responses to MV. In general, there are essentially no data, either from the literature, from epidemiologic studies or from studies of this individual that support a link between the measles vaccine in MMR and autism or "autistic enterocolitis". Ten of the 13 coauthors on the major

paper putting forward this hypothesis have retracted this interpretation of their published data (Murch et al, 2004).

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