

Respondent's Exhibit Z



Centre universitaire de santé McGill
McGill University Health Centre

Les meilleurs soins pour la vie
The Best Care for Life

April 12, 2007

Vincent Matanosky
Assistant Director
United States Department of Justice
Torts Branch, Civil Division
1425 New York Avenue, NW, Rm. 3140
Washington, DC 20005

RE: Michelle Cedillo
DOB: 1994-08-30
DK No 98-916V

Dear Mr Matanoski,

I have reviewed the extensive medical records of Michelle Cedillo and the expert opinions filed by the petitioners.

According to the records, Michelle is the product of a normal pregnancy. She had relatively normal infant development with some delays stated on history during the psychological assessment on July 21, 1997 (Pet Ex 7 at 4) where it is noted that she did not social smile until 4-6 months of age. As noted in the guidelines of the American Academy of Pediatrics, an important warning sign for developmental delay is failure to social smile by age of 3 months. She gained motor milestones more slowly than average and ultimately achieved gross motor milestones at the outer limits of normal. She was noted by the age of 6 months to be above the growth curves for height, weight and head circumference and a note was made at her 15 month visit of a concern of impending obesity (Pet Ex. 8 at 2, 7 and 8).

On December 20, 1995 she received her MMR vaccine. On December 27 she developed high fever which continued until January 1, 1996. On January 5, 1996 she again developed fever and was noted to have a rash by her mother. She was seen at her pediatrician's office and diagnosed with viral illness versus clinical sinusitis (Pet Ex 8 at 2). Eighteen months later, on July 21 1997, after a complete assessment Michelle was diagnosed with severe pervasive developmental delay and intellectual impairment.

Michele is now a 12 year old child with complex medical problems including pervasive developmental delay (autism), intellectual impairment, seizures, inflammatory bowel disease, uveitis, arthropathy and mobility issues. Over the past 12 years she has been seen and followed by many medical specialists and has had numerous investigations.



As part of her medical evaluation, at age 3, Michelle was evaluated by Dr Sudhir Gupta, an Immunologist at UCI Medical Plaza, on 08-06-97. Of note, at that time, it was hypothesized that autism may be related to immune system dysfunction (Stubb et al., 1977, Warren et al., 1986) although more recent large prospective studies do not find any pattern of abnormal immunologic function in autistic children and thus this hypothesis has not been supported in clinical studies (Stern et al, 2005).

After Michelle’s evaluation, Dr Gupta concluded that she had “almost normal immune functions” (Pet Ex 3 at 16). I have reviewed the investigations of the immune system of Michelle Cedillo and agree with Dr Gupta **that the immune system of this 3 year old child was normal for her age.** It should be noted at this time that there were significant limitations on the immune evaluations available to Dr Gupta and the studies done must be interpreted in light of these limitations.

This work up included serum immunoglobulin (antibody) and immunoglobulin subclass levels, vaccine –specific antibody responses, T and B cell enumeration and *in vitro* proliferation studies. She had normal serum immunoglobulin levels, including IgE, and protective antibody titers to diphtheria, tetanus, rubella, polio, and pneumococcus. In addition, her measles IgG levels were protective while her measles IgM levels were negative, thus providing no serological evidence of current or active measles infection (Pet Ex 3 at 7).

The T and B cell enumerations were as follows (Pet Ex 3 at 12). Included in this table are accepted published normal range values age adjusted for Michelle’s age at time of assessment. The stated norms in Pet Ex 3 at 12 are adult and not pediatric values and there are significant age-related variations in normal ranges. (Hannet et al, 1992, Shearer et al 2003, Kawamoto et al 2003, Gasparoni et al, 2003).

Lymphocyte type	Percents %	Absolute numbers (abs#)	Normal range for ages 2-6 yrs % *	Normal range for ages 2-6 yrs abs# *	Interpretation
CD3 (Total T)	66	2120	56-75	1400-3700	normal
CD3/CD4 (THelper)	38	1220	32-47	700-2200	normal
CD3/CD8 (T cytotoxic)	17	550	16-30	490-1300	normal
CD4/CD8 ratio	2.24		1.1-2.9		normal
CD20 (Total B cells)	21	670	21-28	500-800	normal
CD16+ (NKs)	8	260	4-17	130-720	normal

* Normal values taken from Hannet et al 1992, Shearer et al 2003, Kawamoto et al 2003.

Based on accepted normal values for age taken from large cross-sectional studies, the ***T and B cell enumerations of Michelle Cedillo were entirely normal for her age.***

Proliferation studies were also done using both mitogen stimuli and antigen stimuli. Proliferation studies are biological assays with high variability. In children there are no routinely accepted pediatric normal values. Results are generally reported in comparison to control values from control samples obtained on the same day of the test and additional comparison is also made to baseline values from the patient. As none of these were provided in the test results, it is difficult to fully interpret these results. However, using a recently published study of autistic children compared with their age-matched controls it is clear that Michelle Cedillo's proliferation studies with mitogen stimuli fall within the range of both the normal and autistic children (Pet Ex 3 at 13 and Stern et al., 2005).

Mitogens	Patient	Autistic children range	Normal children range	Interpretation
Unstimulated	Not given	175-939	179-732	
PHA	171,064	48,539-274,713	23,254-227,438	Normal range
Con A	116,245	31,698-214,898	27,824-178,167	Normal range
PWM	25,032	5,141-167,508	8,193-124,713	Normal range

Thus within the limitations for interpretation of these test results based on the patients age, ***Michelle Cedillo's lymphocytes showed normal proliferation to mitogens for her age.***

Proliferation studies were also done using mumps, candida, tetanus and PPD antigens (Pet Ex 3 at 14). There are no studies in the extant literature providing normal values for these antigens in children. As Director of the Clinical Immunology Laboratory at the Montreal Children's Hospital I have found a wide degree of variability in this assay system depending upon patient age, health status at the time of the assay, medication use and date and timing of exposures to the various antigens. Examining these results, I would interpret them as positive or negative only. Without age defined standards, it is impossible to do otherwise with any accuracy. Thus I would interpret these results as positive for Mumps, candida and tetanus and negative for PPD, as the 157 value is usually not greater than background in this assay system. Thus in my opinion, these ***results do not indicate immune derangement in this child.***

Finally, immunoglobulin subclasses were also evaluated (Pet Ex 3 at 15). Of the subclasses determined, 2 were defined as elevated compared with stated references ranges, IgG2 at 382 mg/dl and IgG4 at 85 mg/dl. However, when these values were compared with reference values with respect to age (see Rose et al., 2006, Schauer et al, 2003), the ***IgG4 value was within normal for ages 2-5 yrs (range 12-148mg/dl)***. The value for IgG2 listed at 382 was elevated when controlled for age (range 32-311mg/dl). In the extant literature isolated elevation of IgG2 levels has only been reported in patients with periodontal disease (Engstrom et al, 1999). Interestingly, one study of children with

autism reported increased immunoglobulin subclasses IgG1 and IgG4 levels in autistic children compared with their normal siblings. In this study IgG2 levels were not different between patients and controls (Trajkovski et al, 2004) suggesting that subclass variability is an inconsistent finding of dubious relevance in autistic children.

In summary, the immune evaluation of Michelle Cedillo was normal when results are evaluated using age appropriate norms. There is no evidence of immune dysregulation with skewed TH1/TH2 responses. These findings highlight the importance of understanding the normal development of the immune response in children.

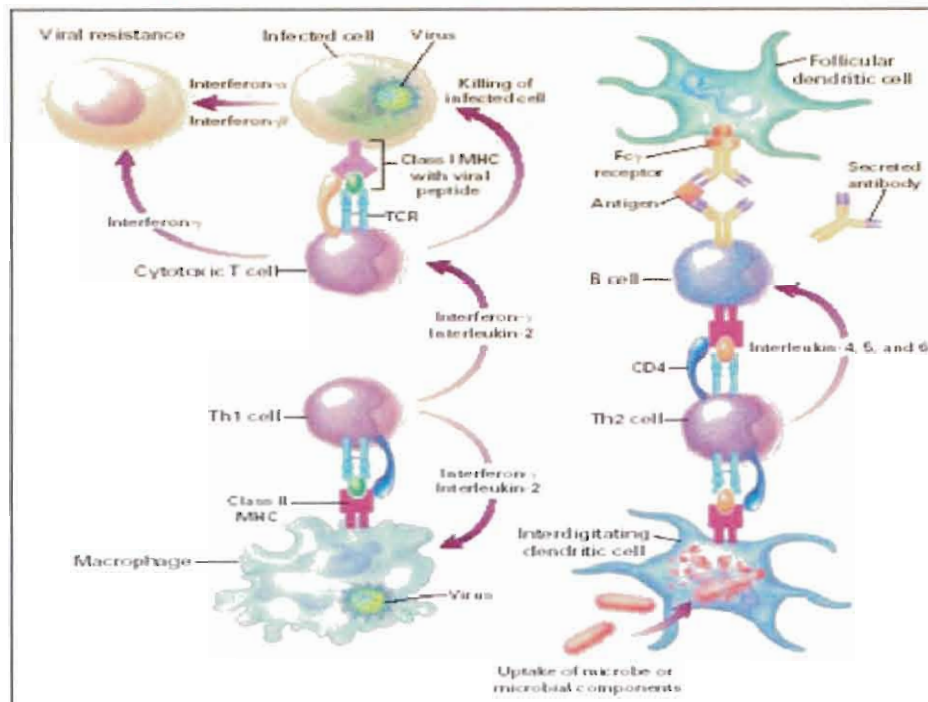
TH1 and TH2 and Immunoregulation.

T-helper 1 (TH1) and T-helper 2 (TH2) cells were first described by Mosmann et al in 1986 and were defined as 2 subsets of T cells producing different cytokines (interferon- γ and interleukin-4 respectively). In general, cytokines are small proteins released from these cells that can direct the functions of other immune cells in the vicinity of an immune response. With work done primarily in mice, it was hypothesized that TH1 and TH2 responses behaved like 2 sides of a coin, where only one response could predominate.

However, our knowledge of T cell biology has expanded tremendously and several distinct TH subsets have been defined. These include the TH3 (producing the cytokine TGF- β), the Tregulatory cell (producing IL10) that functions to turn down both TH1 and TH2 responses and the TH17 cell (producing interleukin-IL17, a proinflammatory cytokine) (Dong, 2006). Thus Dr Byers' statement that "the two systems cross-regulate each other"(Pet Ex 57 at 5) in reference to TH1 and TH2 regulation is not supported by the extant literature. In fact several studies have clearly demonstrated that TH1 and TH2 responses are regulated by TH3, T-regulatory and in some instances by TH17 cells (Kretschmer et al 2005, Miyara et al 2007). Cytokine production and cell to cell contact regulate the formation of the immune response to any given antigen (McGuirk and Mills, 2002).

As depicted in the schematic below, it is generally accepted that TH1 cells are primarily involved in activation of cytotoxic T cell (CTL) responses required for killing of infections (eg viruses) that occur inside the cell (Zanetti and Franchini, 2006). TH2 cells direct the activation of B cells to produce antibodies. It is clear that these functions are not mutually exclusive as during immune responses to viruses and vaccines there is formation of both CTL and antibody responses (Meyer et al, 2007).

Specific Immune Responses to Antigen



In the case of Michelle Cedillo, Dr Byers has proposed that her immune response was skewed with TH2 predominance. TH2-mediated disease is an area of active research as the most common type of TH2 diseases, asthma and allergy, affects up to 30% of the general population (Romagnani, 2004). Allergy and allergic asthma occur when TH2 cells recognize environmental antigens, such as pollens, and activate B cells to produce IgE. An important biomarker of TH2 activation is the formation of serum IgE (Scirica et al, 2007). Several studies have failed to find other easily assayed blood biomarkers that are consistently associated with TH2 responses. TH1 and/or TH2 responses are only defined at present by demonstrating differential cytokine expression in serum or in T cells; Interferon- γ , IL12 among others for TH1 and IL4, IL13 et al for TH2 (Gasparoni et al 2003). Increased circulating IgE is an indirect marker of TH2 activity. It should be noted here that individuals who do not make TH2 responses to pollens, make instead T-regulatory-type responses and are therefore ‘tolerant’ to these environmental antigens (Hawrylowicz and O’Garra, 2005). ***In Michelle’s case her serum IgE level, the only marker of TH2 cell activity assessed, was entirely normal.***

Dr Byers hypothesis (Pet Ex 57 at 7) that Michelle Cedillo had a “dysregulated immune system” that “functionally impairs the two systems responsible for eliminating clearance of pathogens...” is not supported by current literature and understanding of immune responses. TH1 and TH2 immunity are not mutually exclusive and while natural viral infections have been shown to transiently affect immune responses, there is no evidence that this has occurred in this child.

In summary,

1. Interpretation of the immune evaluation utilizing age-appropriate normal ranges shows that Michelle Cedillo had normal immunity at age 3.
2. There is no evidence of ongoing immune dysregulation mediated by persistent measles virus.
3. There is no evidence of TH1/TH2 skewing in the investigations presented. As such the hypotheses of both Dr Byers and Dr Kinsbourne that her Autistic Spectrum Disorder was caused by “mercury-induced immune dysfunction” (Pet Ex 61 at 21) is not supported by the immune laboratory evidence presented.

Taken altogether it is my opinion that Michelle Cedillo suffers from Autistic Spectrum Disorder and has no objective evidence of abnormal immune function.



Christine McCusker MSc, MD, FRCP
Assistant Professor of Pediatrics
Division of Allergy and Immunology
Director, Clinical Immunology Laboratory
Montreal Children’s Hospital of the
McGill University Health Center

References:

1. Stubbs EG and Crawford ML. Depressed lymphocyte responsiveness in autistic children. *J Autism Child Schizophr.* 1977;7(1):49-55.
2. Warren RP, Margaretten NC, Pace NC and Foster A. Immune abnormalities in patients with autism. *J Autism Dev Disord.* 1986;16:189-97.
3. Hannel I, Erkeller-Yuksel F, Lydyard P, Deveys V and De Bruyere M. Developmental and maturational changes in human blood lymphocyte subpopulations. *Immunol Today.* 1992;(6):215, 218.
4. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R et al. Lymphocyte subsets in healthy children from birth through 18 years of age: The Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol.* 2003;112:973-80.
5. Kawamoto N, Kaneko H, Takemura M, Seishima M et al. Age-related changes in intracellular cytokine profiles and TH2 dominance in allergic children. *Pediatr Allergy Immunol.* 2006;17:125-133.
6. Gasparoni A, Ciardelli L, Avanzini A, Castellazzi AM, et al. Age-related changes in intracellular TH1/TH2 cytokine production, immunoproliferative T lymphocyte response and natural killer cell activity in newborns, children and adults. *Biol Neonate.* 2003;84:297-303.
7. Stern L, Francoeur MJ, Primeau MN, Sommerville W, Fombonne E and Mazer BD. Immune function in autistic children. *Ann Allergy Asthma Immunol.* 2005;95:558-565.
8. Rose MA, Schubert R, Kujumdshiev S, Kitz R and Zielen S. Immunoglobulins and immunogenicity of pneumococcal vaccination in preschool asthma. *Int J Clin Pract.* 2006;11:1425-1431.
9. Schauer U, Stemberg F, Rieger CHL, Borte M et al. IgG subclass concentrations in certified reference material 470 and reference values for children and adults determined with the binding site reagents. *Clin Chem.* 2003;49:1924-1929.
10. Engstrom PE, George M, Larsson P, Lally ET et al. Oral and systemic immunoglobulin G-subclass antibodies to *Actinobacillus actinomycetemcomitans* leukotoxin. *Oral Microbiol Immunol.* 1999;14:104-108.
11. Trajkovski V, Ajdinski L and Spiroski M. Plasma concentration of immunoglobulin classes and subclasses in children with autism in the Republic of Macedonia: Retrospective study. *Croat Med J.* 2004;45:746-749
12. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA and Coffman ML. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol.* 1986;136(7):2348-57.
13. Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. *Nat Rev Immunol.* 2006;6(4):329-33.
14. Kretschmer K, Apostolou I, Hawiger D, Khazaie K, et al. Inducing and expanding regulatory T cell populations by foreign antigen. *Nature Immunol.* 2005;6:1219-1227.

15. Miyara M and Sakaguchi S. Natural regulatory T cells: Mechanisms of suppression. Trends in Molec Med. 2007;DOI: 10.1016/j.molmed.20070103. *in press*.
16. McGuirk P and Mills KHG. Pathogen-specific regulatory T cells provoke shift in the TH1/TH2 paradigm in immunity to infectious diseases. Trends in Immunol. 2002;23:450-455.
17. Zanetti M and Franchini G. T cell memory and protective immunity by vaccination: is more better? Trends in Immunol. 2006;27:511-517
18. Meyer CU, Zepp F, Decker M, Lee M, Chang SJ, Ward J, Yoder S, Bogaert H, Edwards KM. Cellular immunity in adolescents and adults following acellular pertussis vaccine administration. Clin Vaccine Immunol. 2007;14(3):288-92.
19. Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression of both? Immunol. 2004;112:352-363.
20. Scirica CV, Gold DR, Ryan L, Abulkerim H et al. Predictors of cord blood IgE levels in children at risk for asthma and atopy. J Allergy Clin Immunol. 2007;119:81-88.
21. Hawrylowicz CM and O'Gara A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. Nature Rev Immunol. 2005;5:271-283.