Written Supplement to Oral Testimony at the Hearing of the
Government Reform Committee,
Congress of the United States
US House of Representatives
by

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June 19, 2002

Mr. Chairman and Honorable Member of the Committee, much has happened to forward our knowledge of autism spectrum disorders (ASD) since I last spoke to you one year ago. To that end I am encouraged. But there remains so much more to learn, and even more to do for the families whose lives have been permanently altered by this silent epidemic. I want to thank Chairman Burton for his leadership. Your battle to bring the challenging issues of autism before the Congress, poignantly demonstrate the power of a grandfather's love for his family. I am equally impressed by the efforts of Dr Dave Weldon. He remains a trusted friend, fellow physician and Representative for my home district in Florida.

Autism is clearly not any single entity, nor does it have simplistic genetic or epidemiological characteristics. Rather, it represents a rather broad spectrum of clinical disorders which share behavioral and delayed-development features. Autism and its related entities are characterized by: delayed neurodevelopment, lack or inappropriate use of language, stereotypical repetitive behaviors, and social withdrawal. The various clinicians and researchers associated or affiliated with ICDRC have been involved in treating and/or describing this disorder from its biological roots, as opposed to the genetic and psychiatric perspectives. We have medically evaluated and treated over 1500

children with autism related disorders. Therefore, the insights we have contribute primarily to an understanding of the immunology and toxicology of this condition.

The changes since last year in the level of national attention for autism are well reflected by events in my life during the last few weeks. On June 4th, Congressman Weldon and I met with the Deputy Secretary of HHS, Claude Allen in Chairman Burton's office to discuss both recent clinical findings and the state of the autism epidemic. Then ICDRC entered into collaboration agreements with Robert Wood Johnson Medical Center, Washington University Medical School, and Wake Forrest University to further define the immunological and toxicological disorders common in autism. And last Tuesday an ABC news crew spent the entire day filming at both my office and home. Those segments will air tonight and tomorrow on the ABC evening news.

With this new public and academic awareness of the epidemic in childhood developmental disorders in mind, where has the last year's investigations taken our understanding of both Thimerosal and MMR as they relate to autism? In July of 2001, I presented the ICDRC data on mercury burden and autoimmunity to the IOM (page 47, **Immunization Safety Review: Thimerosal-Containing Vaccines and Neurodevelopmental Disorders**, 2001). It doesn't seem the IOM understood my recommendations based on that data, so it warrants some degree of explanation here.

First, however, there is a fundamental flaw in the analysis process of vaccine safety. The IOM has undertaken the process of drawing conclusions regarding separate pieces of the actual schedule when they are an integrated event in an individual child's life.

I presented 221 children with ASD who showed a significant - 500% - on average greater mercury burden when compared to neurologically normal controls. The study was based on routine heavy metal provocation challenge testing similar to that published in *Environmental Health Perspectives* that same year. I did not try to infer a direct tie to thimerosal. Rather, it was apparent some possible foundational problem in the

metabolism of heavy metals was present in the autistic population. This observation could represent a significant predisposing factor in their vulnerability to mercury when used as a perservative – a point the IOM did not mention. It is also consistent with research regarding sulfur depletion in the presence of persistent viral infections. The literature is replete with reference in the case of HIV* and specific to autism as published by Dr Rosemary Waring. She has found marked renal loss of sulfur in autism.*

But most concerning to me in the Institute's treatment of the mercury problems, was the almost complete absence of regard for the compounding effect of thimerosal on pre-existing mercury levels. The NHANES study from CDC had already established perhaps one in ten children is born to mothers with elevated mercury burden.

Prevalence:

Various studies provide data that there are greater numbers of children with autism than previous suspected. Recently, the Congressional Reform Committee, held hearings where there was broad consensus that autism spectrum disorders (ASD), now represent an epidemic of neurodevelopmental problems for our youth. Various **recent studies place the prevalence at 57 to 67/10,000 children (Scott, 2002 & Bertrand, 2001**), although older literature places the prevalence at 10/10,000 (1/1,000). However, this deceptively under estimates the problem for males. Boys suffer from autism at a four to 10 fold greater frequency than girls. So the actual problem for the male offspring in this country is more accurately represented as 100/10,000 (or greater). **The 1997 US Census of disability reported 2.4% of children ages five and under suffer from developmental delays** – clearly many of these are ASD related issues. Data from California further reveals the rate of growth in ASD is doubling every four years.

Using simple math – we appear to be on the Titanic of child development:

1:149 US children according to the CDC, have autism. That is the statistic for Brick NJ, but it was implied by CDC to be consistent with the likely general stats.

> ***Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation.***
> *Pediatrics 2001 Nov;108(5):1155-61*
> *Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P.*
>
> *National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia 30341, USA. jbertrand@cdc.gov*
> *RESULTS: The prevalence of all autism spectrum disorders combined was 6.7 cases per 1000 children (1:149). The prevalence for children whose condition met full diagnostic criteria for autistic disorder was 4.0 cases per 1000 children, and the prevalence for PDD-NOS and Asperger disorder was 2.7 cases per 1000 children. Characteristics of children with autism in this study were similar to those in previous studies of autism. CONCLUSIONS: The prevalence of autism in Brick Township seems to be higher than that in other studies, particularly studies conducted in the United States, but within the range of a few recent studies in smaller populations that used more thorough case-finding methods.*

1:149 = one child per 68 homes (assuming 2.2 children per family)

And form the US Census Bureau

Population, 2001 estimate 284,796,887

Persons under 5 years old, percent, 2000 6.8%

Persons under 18 years old, percent, 2000 25.7%

If the current epidemiology of autism is correct, then it will affect approximately 1% of boys under 18, or an estimated 364,540, and a further approximately 60,000

girls would be affected. This is considerably less than the one million figure reported in the recent Time Magazine cover story, but probably far more accurate.

Economic Impact:

While no precise studies have attempted to look at the cost of correcting the biological problems associated with ASD, at least on report from England places the custodial costs of ASD in the range of $3-4 million per child per lifetime, with a societal cost that would likely be three times the individual cost.

> Autism 2001 Mar;5(1):7-22
> **The economic impact of autism in Britain.**
> Jarbrink K, Knapp M.
> Institute of Psychiatry, London, UK.
>
> Little is known about the economic impact of autism. This study estimated the economic consequences of autism in the United Kingdom, based on published evidence and on the reanalysis of data holdings at the Centre for the Economics of Mental Health (CEMH). With an assumed prevalence of 5 per 10,000 *(a gross underestimate)*, the annual societal cost for the UK was estimated to exceed l1 billion *(likely 110 billion)*. The lifetime cost for a person with autism exceeded £2.4 million. The main costs were for living support and day activities. Family costs account for only 2.3 percent of the total cost, but a lack of relevant information limited our ability to estimate these costs. **Minor improvements in life outcome for people with autism could substantially reduce costs over the lifetime**.

The cost of education, medical care, and therapies for behavioral and physical symptoms is staggering. Many of our families report having paid $50,000 per year to care for their child. IDEA allows up to $35,000/year for education of children with autism. So much of this burden is already being carried by the Federal and State programs which provide for disabled children. Custodial care for autism can exceed $100,000/year. The public education system is literally swamped with children. Any survey of public educators will quickly reveal the suddenness and magnitude of the ASD problem. They lack the therapists and trained special educators to deal with the problem, so children with severe disorders receive nominal meaningful intervention. The further loss of potential earnings form the ASD children who will likely not be self-supporting are impossibly large to calculate meaningfully. Many parents must quit working to care for the child as well. We, as a nation, are therefore paying and will continue to pay an enormous price for this epidemic.

ICDRC estimates the minimal cost in present value, to care for those 420,000 existing children with autism is $1,260,000,000,000 (based on $3million/lifetime and 420,000 children affected). So a little over a $1 trillion in the next 50 years would be required if we stopped creating new cases today. Because autism is doubling every four years, this is likely an overly conservative estimate. The societal cost could easily be $3-4 trillion.

Biological Evidence of Causality:

The data will show there is sufficient cause for concern and abundant published findings that the causal relationship of MMR to ASD does not represent a narrow view held by radical or renegade physicians. Rather it is sound peer-reviewed science, which, while currently not widely accepted, represents a plausible hypothesis consistent with our observations and the totality of the data. Unfortunately, the present objections to the data are largely based on conclusions drawn from epidemiological studies. The data

must be evaluated in its entirety, rather than critiqued bit by bit as it has been. However, as a clinician treating hundreds of children with specific & measurable biological disorders – I draw very little comfort from the conclusions of epidemiologists. Nor does it help me explain or treat the child's inflammatory bowel disease or the autoimmunity to vital brain components. So what I will present here today is a definable clinical disorder, in which children present with antibodies to a variety of brain components, inflammatory bowel disease, heavy metal burdens, often accompanied by seizures, skeletal maturation delay and a variety of significant biochemical abnormalities. The children I treat have symptoms consistent with encephalopathy with autistic features.

We are in the process of collecting data and analyzing the trends in our patient population. The two cases I will present here represent very early data. We have now accumulated simultaneous autoimmune, immune studies and viral polymerase chain reaction studies on blood, spinal fluid and intestinal biopsies. These are combined with comprehensive biological studies. As yet, there are no controls for the viral spinal fluid data, but the immunological data does have controls. What these two cases mean for the rest of the population of children with autism will have to wait for larger studies, reproducibility and necessary controls.

Case Presentations:

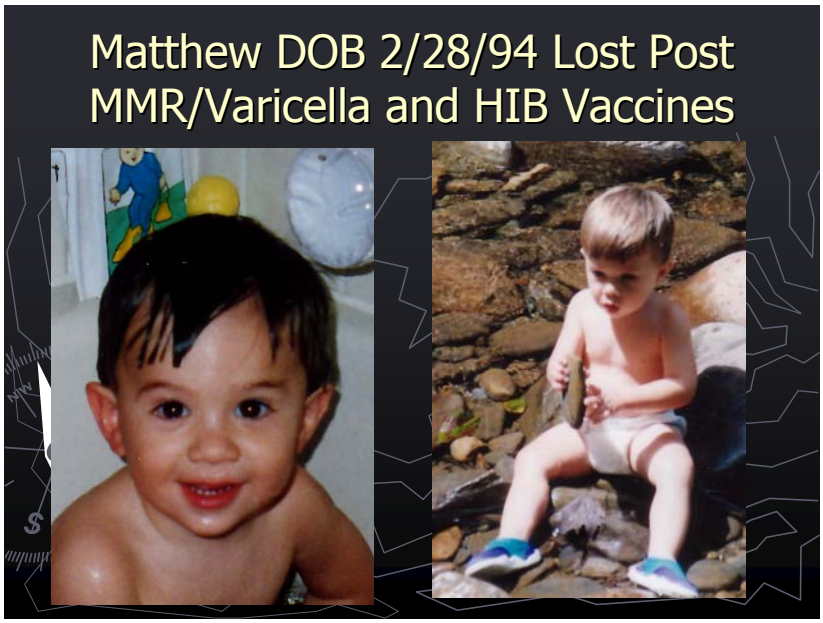
Case 1. Matthew, my son, seems very typical of many children I have examined over the past 5 years. He shares similar historical events and laboratory data with as many as 80% of our 1500 patients. He presently is age 8 and went to term without complication in pregnancy and had an uncomplicated labor and delivery. He presented with an entirely normal first 7 months. At the end of that period he self-weaned and standard formula was tried. This resulted in reflux and vomiting, so he was changed to predigested formulas with significant reduction in symptoms. The pediatrician noted slight delay in ambulation at 12 months, but in line with maternal developmental patterns. He had a protracted otitis media which required tube placement by 10 months and extended courses of antibiotics.

By 11 months he was seen in the ER for an acute febrile event not accompanied by seizures. It responded to IV antibiotics and outpatient treatments. Near 15 months he was seen for routine care and vaccinations. He was noted to be on track and developing normally. He received MMR, HIB and Varicella vaccinations at that visit. Shortly after that he developed tantrums and bizarre behaviors. Then he developed diarrhea and hyperactivity accompanied by a new symptom – night terrors. With the introduction of essential amino acids and taurine these symptoms improved somewhat for about 8 to 12 months. He then began slipping with increased hyperactivity and unusual language and behaviors. By age three he was diagnosed as having pervasive developmental delays and tested at the lowest percentile for function in all areas. He was started in therapies and improved somewhat. On his 4th birthday the original Wakefield paper was published and at nearly the same time, Matt received his MMR booster. (He received the full recommendations of the AAP for vaccinations during the mid to late 1990s). Shortly thereafter we noted staring spells as did the special needs teacher in his title H program. The neurologist diagnosed seizures and tried several medications unsuccessfully. His diarrhea returned and his behavior declined. Several months later we learned about gluten and casein free diets, secretin and IVIG. After a variety of studies confirmed autoimmunity to his brain, Matthew was begun on IVIG at the suggestion of two department chairs of immunology at different medical schools. The results were dramatic, with improvement in behavior and bowel dysfunction which had become explosive bouts with daily soiling past diapers.

The process of regression was not understood by Matthew's pediatrician or any of us in his family. Typically, it was variously dismissed as the result of the terrible two's, having an older sister, being a boy – "they are slower than girls you know", several ear infections, food allergies, or an attention deficit hyperactivity disorder.

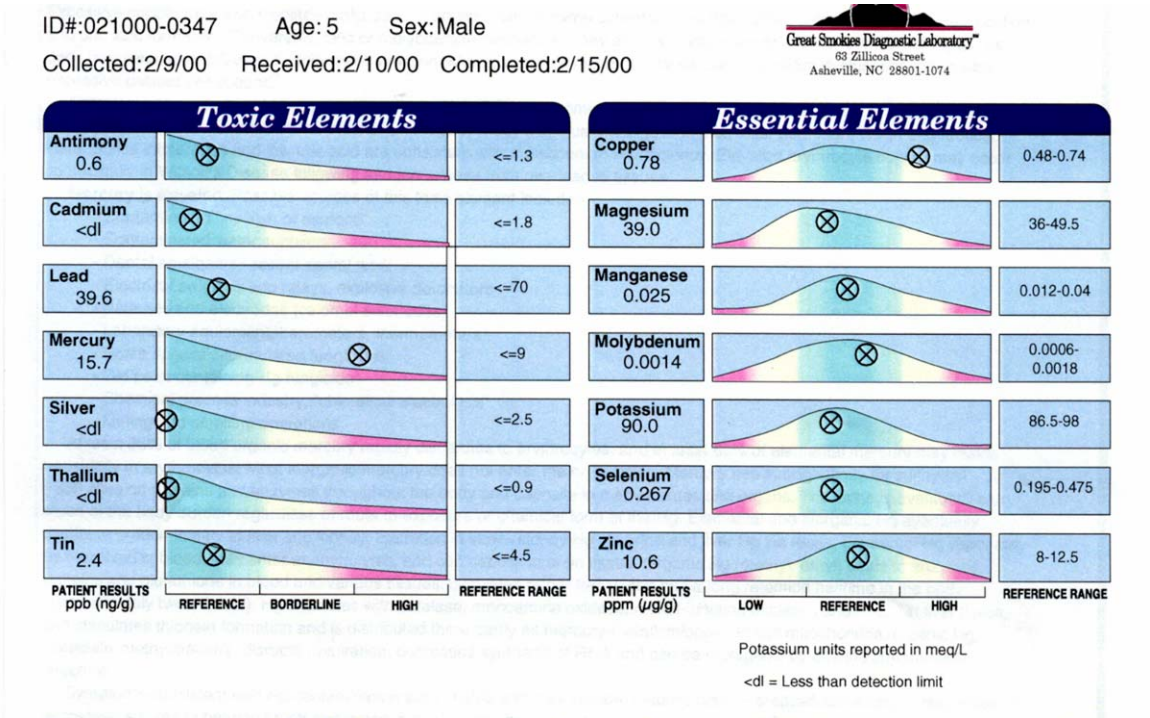
If we are to believe the experts from the IOM and Vaccine Safety Committee of CDC, my son's autism was a coincidental event, and these double hit MMR events are of no consequence, because MMR has nothing to do with autism. A few years ago, Dr. Neal

Halsey, the eminent professor of vaccine safety, told the listening audience of CNN that it was natural for me to want to blame something for my son's autism, but MMR was unquestionably not part of either the timing or autoimmune profile observed. I believe, medicine lacks the luxury of such amazing confidence. However, it seems extraordinarily improbable that his autoimmune encephalopathy and seizures are not MMR related. A review of his lab data paints an unmistakable picture, recognizable to any skilled clinician. **I choose to share the details of my son's medical history, so that those who continue the refrain – "there is no data" might know they are wrong – data exist – and it is compelling.**



(Photo set 1. Reflects excellent & happy eye contact at age 12 months but loss of contact at age 18 months where he could only repetitively bang the rock – language lost at this point).

Review of Labs:



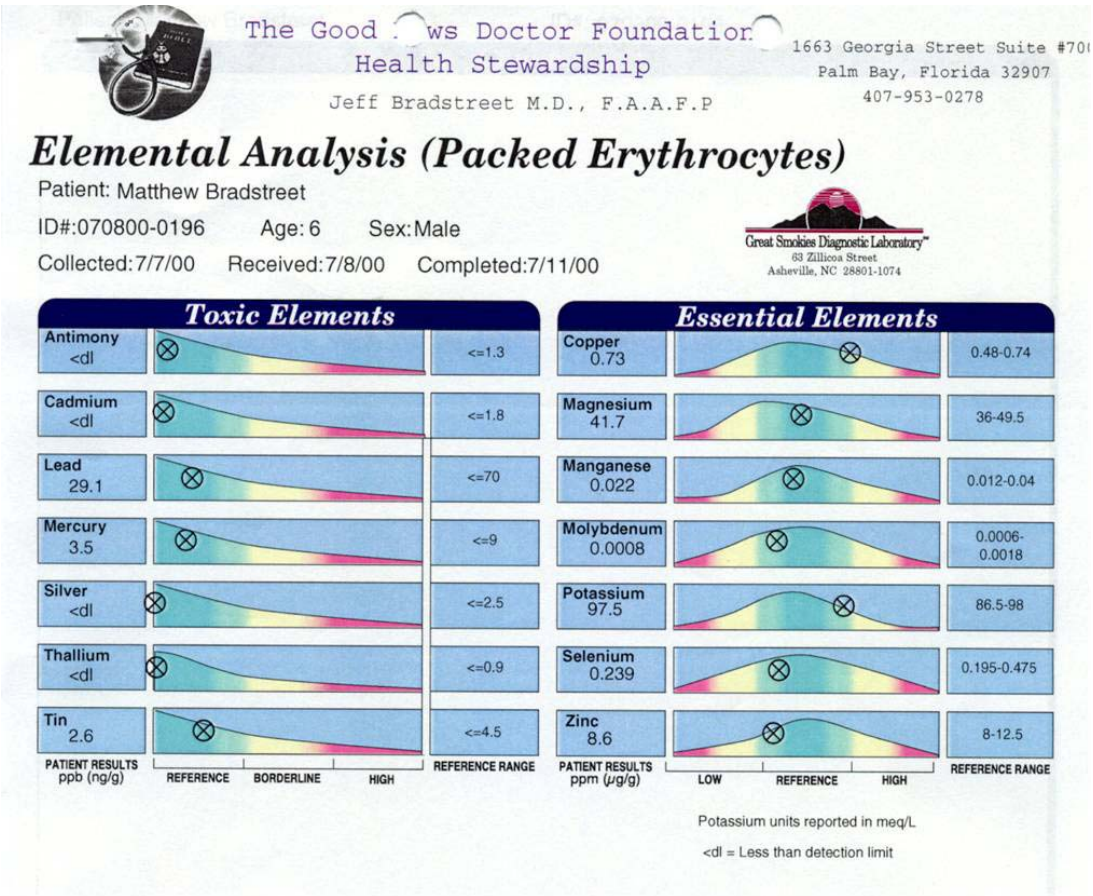
(Figure 1. Above) First heavy metal and nutritional mineral study of packed erythrocytes prior to his 5th birthday, at the end of his vaccine schedule.

DOCTOR'S DATA Inc. Doctor's Data, Inc. P.O. Box 111 West Chicago, Illinois 60186-0111 CALL TOLL FREE (800) 323-2784 James T. Hicks, M.D., Ph.D., FCAP Medical Director CLIA ID # 14D0646470, Medicare Provider # 148453	**Urine Toxic Elements** CHEMET/X2D Lab #: 99572-0088 ET Patient: Matthew Bradstreet Age: 6 Sex: Male Doctor: James Jeff Bradstreet, Acct #: 24503 c/o: Collection Type: Random Collection Date: 21 Jul 2000 Time: Vol:.8L Date In: 25 Jul 2000 Date Out: 28 Jul 2000

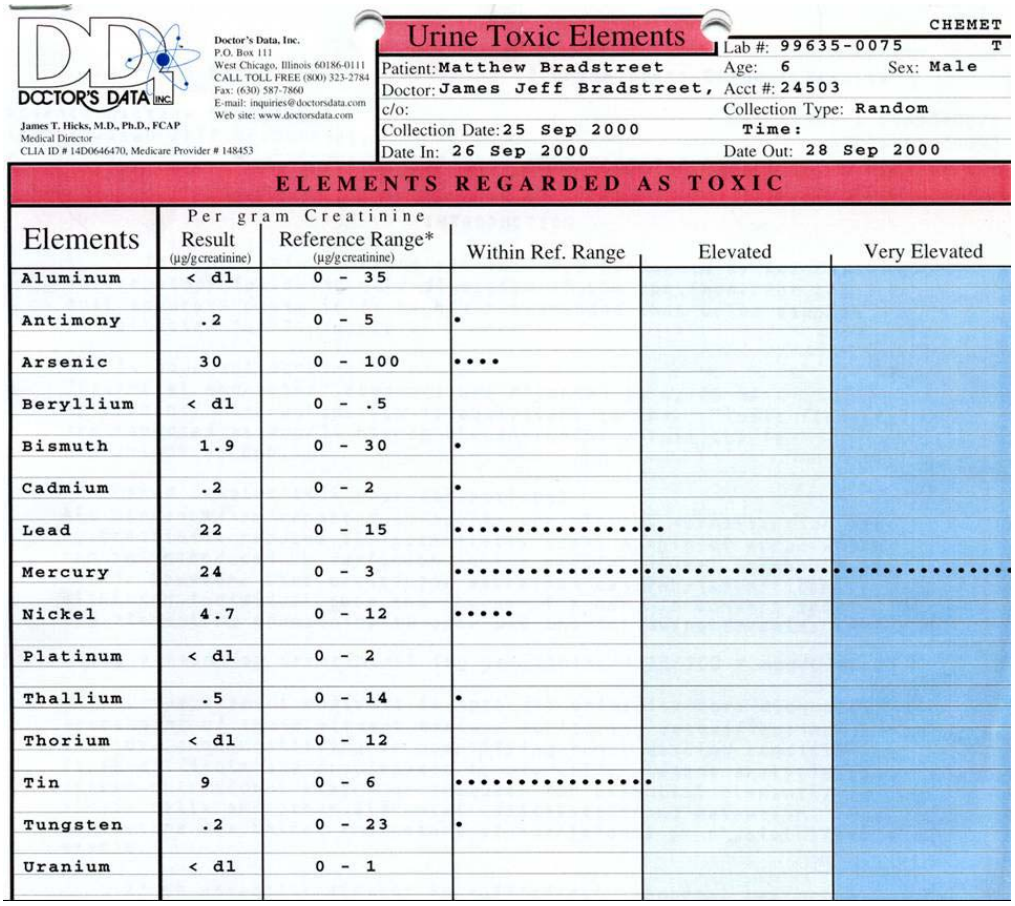
ELEMENTS REGARDED AS TOXIC

Elements	Per gram Creatinine Result (µg/g creatinine)	Reference Range* (µg/g creatinine)	Within Ref. Range	Elevated	Very Elevated
Aluminum	< dl	0 - 35			
Antimony	.2	0 - 5	•		
Arsenic	43	0 - 100	••••••		
Beryllium	< dl	0 - .5			
Bismuth	2.7	0 - 30	•		
Cadmium	.2	0 - 2	•		
Lead	27	0 - 15	••••••••••••••••••	•••••	
Mercury	11	0 - 3	•••••••••••••••	•••••••••••••••	••••••••••••
Nickel	3.3	0 - 12	••••		
Platinum	< dl	0 - 2			
Thallium	.4	0 - 14	•		
Thorium	< dl	0 - 12			
Tin	9.2	0 - 6	•••••••••••••••	•	
Tungsten	.1	0 - 23	•		
Uranium	< dl	0 - 1			

(Figure 2. First chelation challenge for determination of heavy metal burden. For this study 10 mg per kilogram of body weight of DMSA – Chemet-Sanofi were given to three times daily for three days. Urine was collected on the morning of the 4th day. Matthew has no amalgams – mercury filings and ate fish only occasionally. His mother was also tested and had minimally detectable levels of mercury in both red blood cells and after a chelation provocation challenge test.)



(Figure 3. Repeat packed erythrocyte level after several rounds of DMSA - Chemet, showing significant improvement. The first course of DMSA used the FDA recommendations for lead which is a 19 day course. There were no apparent side-effects during the chelation.)



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James T. Hicks, M.D., Ph.D., FCAP
Medical Director
CLIA ID # 14D0646470, Medicare Provider # 148453

Urine Toxic Elements CHEMET

Patient: Matthew Bradstreet | Lab #: 99635-0075 T
Age: 6 | Sex: Male
Doctor: James Jeff Bradstreet, | Acct #: 24503
c/o: | Collection Type: Random
Collection Date: 25 Sep 2000 | Time:
Date In: 26 Sep 2000 | Date Out: 28 Sep 2000

ELEMENTS REGARDED AS TOXIC

Elements	Per gram Creatinine Result (µg/g creatinine)	Reference Range* (µg/g creatinine)	Within Ref. Range	Elevated	Very Elevated
Aluminum	< dl	0 - 35			
Antimony	.2	0 - 5	•		
Arsenic	30	0 - 100	••••		
Beryllium	< dl	0 - .5			
Bismuth	1.9	0 - 30	•		
Cadmium	.2	0 - 2	•		
Lead	22	0 - 15	••••••••••••••••		
Mercury	24	0 - 3	••••••••••••••••	••••••••••••••••	••••••••••••••••
Nickel	4.7	0 - 12	•••••		
Platinum	< dl	0 - 2			
Thallium	.5	0 - 14	•		
Thorium	< dl	0 - 12			
Tin	9	0 - 6	••••••••••••••••		
Tungsten	.2	0 - 23	•		
Uranium	< dl	0 - 1			

(Figure 4. Repeat DMSA urine provoked heavy metal study showing marked increase in mercury excretion despite 6 months of treatment indicating a very significant total body burden of mercury. In this study, we actually see a significantly greater level of mercury. This is presumably secondary to mobilization of Hg and redirection to the kidney where it could be eliminated.)

But intermixed with these studies were investigations for inflammatory bowel disease which did demonstrate grade II nodular hyperplasia of the terminal ileum and eosinophilic colitis and enteritis. He had autoantibodies to MBP detected about a year after his second MMR vaccine.

ICDRC

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Report for Measles Virus Detection

Client information:

Name:	Matthew Bradstreet
Patient Identification	BL5606
Date of Birth:	28/02/1994
Lab Number:	265

TEST - MEASLES VIRUS DETECTION

RNA was extracted from the ileal biopsy and measles virus was detected using a reverse transcriptase quantitative PCR procedure (TaqMan RT-PCR).

The detected measles virus per ng of RNA:F Gene: 1.0 X 10^3 copies/ng total RNA

Result: **Positive for Measles Virus**

Approved by: _____ Date: 02/02/[illegible]

Prof. J. O'Leary

(Figure 5. Represents the results of viral Fusion gene (F gene) investigations of the terminal ileum taken on evaluation at the Royal Free Hospital by Dr Tomson in August of 2000.

ICDRC

Date: March 20, 2002

Subject Name: **Matthew Bradstreet** DOB/Age: 2/28/94
Address: c/o Jeff Bradstreet, MD
1663 Georgia Street, Suite 700, Palm bay, FL 32907
Referring Physician: Dr. Jeff Bradstreet, MD

LABORATORY RESULTS (For Investigational Use Only)

SPECIMEN: ☐ Serum ☑ **CSF** ☐ Other Specimen Date: 3/7/02

Test	Result
1. Myelin Basic Protein (MBP) Antibody (Screened at 1:26 dilution of CSF)	**Positive** (weak reaction)
2. Neuron-axon Filament Proteins (NFP) Antibody (Screened at 1:26 dilution of CSF)	Negative
3. Measles Virus (MV) IgG Antibody (Screened at 1:5 dilution of CSF)	0.4 Unit (low level signal)
4. Measles-Mumps-Rubella (MMR) Antibody (Screened at 1:8.5 dilution of CSF)	Negative
5. Human Herpesvirus-6 (HHV-6) IgG (Screened at 1:5 dilution of CSF)	Negative (below detection limit)

Comment:
This child's CSF shows a mild sign of autoimmune reaction to brain myelin sheath. A low level signal for measles virus antibody was also detected.

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Report for Measles Virus Detection

Client information:

Name:	Matthew Bradstreet
Patient Identification	BL5606
Date of Birth:	28/02/1994
Lab Number:	265

Sample Information:

Date of Sample Collection: 07/03/2002

Date of Sample Receipt: 13/03/2002

Type of Sample(s) Received: Spinal Fluid with RNA Later

Condition of Sample(s): Satisfactory

TEST - MEASLES VIRUS DETECTION

RNA was extracted from spinal fluid and measles virus was detected using a reverse transcriptase quantitative PCR procedure [TaqMan RT-PCR].

The detected measles virus per ng of RNA: F Gene: 6.16 x 10^6 copies/ng total RNA.

Result: **Positive for Measles Virus.**

Approved by: Prof. John O'Leary

Date: 17/04/2002

Summary of Major Abnormalities in Matthew:

- Milk allergy early in life
- Multiple ear infections
- Transient gait abnormality up until about one year. Was this mercury related?
- Rapid decline after each MMR or combination of vaccines with MMR
- Autoimmunity to Myelin Basic Protein (the insulation of the central nervous system).
- Seizures
- Immune Deficiency with protracted low lymphocyte levels
- Inflammatory Bowel Disease
- Persistent Measles Virus genome in that inflammatory bowel disease
- Persistent Measles Virus in circulating monocytes
- Persistent Measles Virus in genome in spinal fluid
- Antibodies to Measles Virus in spinal fluid
- Autoantibodies to Myelin Basic Protein in spinal fluid
- Elevated ammonia
- Low sulfate with resultant high mercury due to a loss of glutathione and cysteine

So, in my child, what would a reasonable clinician conclude for the medical diagnoses? Autism? Certainly not, unless they believed the hypotheses of Wakefield, Singh and a handful of others who are arguing as I am, that what we have come to call autism – in fact represents a new disorder of immune, viral and toxic origin.

About the only question left to answer is – did the viral persistence cause the condition or did the condition cause the viral persistence? In part, we need to consider the toxicity of thimerosal and Matthew's early gait disorder. Thimerosal becomes a neurotoxin as soon as it dissociates and liberates ethylmercury. The levels of mercury obtained in the vaccine – likely combined with environmental mercury from various sources to precipitate the early motor/coordination/gait problems. Tiddelbaum and colleagues from the University

of Florida have published their findings regarding early movement disorders as a predictor of future risk of autism. This may well be an association with the subtle effects of mercury, although that was not their conclusion. I believe we can the inherent "chicken or egg question", but I want to present another case to establish that my son's condition is not an isolated event.

Case 2. I presented this child to this committee last year. Scott was born 7/25/95. For brevity sake I will comment only that numerous documentations of his early development established no abnormalities at all. Shortly after receiving the MMR vaccine Scott became fussy and lost eye contact and then developed diarrhea and behavioral and developmental regressions. On 2/20/99 we obtained a serum specimen from Scott for evaluation at the University of Michigan, College of Pharmacy, Neuroimmunological Research Laboratory of Dr. V.K. Singh. The 2/20/99 serum underwent testing for autoantibodies to myelin basic protein (a component of the central nervous system), which were found to be positive at a dilution of 1:400 - (Strongly positive range). Scott has had repeated spinal fluid analysis which will be presented here. He has also had intestinal biopsies and blood work for the detection of measles virus F gene – all of which are positive. Scott's parents have filled a claim in the Federal Court of Claims alleging the MMR vaccine precipitated their child's autoimmune encephalopathy – which like my son's – has autistic features. They, as parents, are also not reassured by epidemiological studies or arguments from the public health officials claiming MMR cannot cause the disorder their son has.

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Report for Measles Virus Detection

Client information:

Name:	Franklin Scott
Patient Identification	0001667-484
Date of Birth:	07/02/1995
Lab Number:	476

Sample Information:

Date of Sample Collection: 29/01/2002

Date of Sample Receipt: 01/02/2002

Type of Sample(s) Received: Frozen Whole Blood

Condition of Sample(s): Satisfactory

TEST - MEASLES VIRUS DETECTION

RNA was extracted from whole blood and measles virus was detected using a reverse transcriptase quantitative PCR procedure [TaqMan RT-PCR].

The detected measles virus per ng of RNA: F Gene: 6.21 x 10^0 copies/ng total RNA.

Result: **Positive for Measles Virus.**

Approved by: _____ Date: 12/04/2002
Prof. John O'Leary

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Report for Measles Virus Detection

Client information:

Name:	Franklin Scott
Patient Identification	0001667-484
Date of Birth:	07/02/1995
Lab Number:	476

Sample Information:

Date of Sample Collection: 29/01/2002

Date of Sample Receipt: 01/02/2002

Type of Sample(s) Received: T-ileum biopsy

Condition of Sample(s): Satisfactory

TEST - MEASLES VIRUS DETECTION

RNA was extracted from the ileal biopsy and measles virus was detected using a reverse transcriptase quantitative PCR procedure [TaqMan RT-PCR].

The detected measles virus per ng of RNA: F Gene: 3.56 x 10^2 copies/ng total RNA.

Result: **Positive for Measles Virus.**

Approved by: Prof. John O'Leary Date: 12/04/2002

ICDRC

Date: September 4, 2001

Subject Name: **Scott P. Franklin** DOB/Age: 7/2/95

Address: c/o Dr. Jeff Bradstreet, 1663 Georgia Street, Suite 700, Palm Bay, Florida
Referring Physician: Dr. Jeff Bradstreet, M.D., Palm Bay, FL 32907

LABORATORY RESULTS (For Investigational Use Only)

SPECIMEN: Serum/**CSF**/Other Date: 3/31/01

Test	Result
1. Measles-Mumps-Rubella (MMR) Antibody (CSF tested at 1:8.5 dilution)	**Positive***

COMMENT:

*This result indicates an inappropriate or abnormal immune reaction to MMR, which appears to be related to brain autoimmunity in autistic children. According to our other research, the MMR antibodies recognize measles subunit, but not the mumps or rubella subunit, of this multivalent vaccine.

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Tel: (435) 797-7193 Fax: (435) 797-2766

Date: April 12, 2001

Subject Name: **Scott P. Franklin** DOB/Age: 7/2/95

Address: c/o Dr. Jeff Bradstreet, 1663 Georgia Street, Suite 700, Palm Bay, Florida
Referring Physician: Dr. Jeff Bradstreet, M.D., Palm Bay, FL 32907

LABORATORY RESULTS (For Investigational Use Only)

SPECIMEN: Serum/CSF/Other Date: 3/31/01

Test	Result
1. Myelin Basic Protein (MBP) Antibody (Positive at two dilutions: 1:25 and 1:50 dilution)	**Positive***
2. Neuron-axon Filament Proteins (NFP) Antibody (Negative at two dilutions: 1:25 and 1:50 dilution)	Negative
3. Measles Virus (MV) IgG Antibody (Detectable at 1:6 and 1:11 dilutions)	0.8 Units **(Positive)****
4. Human Herpesvirus-6 (HHV-6) IgG Antibody (Undetectable at 1:6 and 1:11 dilutions)	0 Units (Negative)

COMMENT:

*This is a sign of autoimmunity to brain myelin proteins but not to neurofilament proteins, suggesting autoantibody specificity for the brain myelin sheath. Note that NFP autoantibodies were tested as a control for MBP autoantibodies.

**Antibodies to measles virus were present but antibodies to human herpesvirus-6 (HHV-6) were absent; this indicates a measles virus infection. Note that HHV-6 antibodies were measured as a control for measles virus antibodies.

To conclude, there is a possibility of an autoimmune reaction to brain myelin sheath, presumably secondary to a measles infection.

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Conclusions regarding these two cases:

The implications of these findings could have incredible potential impact on public health policy and the future acceptance of voluntary vaccines by parents for their children. We desire safer vaccines and safer administration of vaccines, but we fear a lack of government response to the concerns of researchers and parents will result in lowered overall immunity to numerous preventable disorders, because parents will reject some of the vaccines in their current forms. The request for a safer MMR vaccine was also presented by Imani and colleagues at the Division of Clinical Immunology, Department of Medicine, The Johns Hopkins University School of Medicine, Asthma and Allergy Center (Clin Immunol 2001 Sep;100(3):355-61). So, we do not feel alone in our understanding of the apparent immunological flaws of the current trivalent vaccine.

These data are also public knowledge and have been presented at numerous professional and public forums, as well as through publication in mainstream medical literature, eg *Pediatrics, The Journal of Pediatric, The British Medical Journal – Molecular Pathology, The American Journal of Gastroenterology,* and recently in a press release from *the American Society of Microbiology*. Historically, high titer measles vaccine caused more mortality than expected due to the induction of immune deficiency (Halsey 1993). This caused a reversal of policy and high titer MV vaccines no longer exist. The nature of mass vaccination programs are in effect an ongoing open-label experiment. No study can predict the long-term and subtle effects of a vaccine adequately prior to introduction to a group as large as most of the population of our planet.

Unfortunately, and as true of many new discoveries in medicine, the initial reactions are that of skepticism or rejection. We have seen this historically with *H.pylori* and peptic ulcer disease, as well as during the emerging literature on AIDS and HIV. Eventually, the early observations in these disorders were proven accurate, medicine adapted and acceptance became universal. We believe the same is true for this literature which will be summarized below, despite the present political incorrectness of the findings.

What do we know so far:

1) MV wild type persists in seemingly normal individuals, although I would have preferred a more in depth study of the histories obtained from autopsy studies.

2) Therefore the mere presence of MV (even vaccine strain) is not enough evidence for us to claim causality, although it is definitely not reassuring to find it in the CSF of children with encephalopathy, or in the intestines of f children with inflammatory bowel disease. MV is known to cause encephalopathy and as found in the study from the Mayo Clinic – it is also a risk factor for inflammatory bowel disease.

3) We have shown - through Dr Singh's efforts that the children are reacting to the virus (immune response) - which are not seen in controls. The response is to Myelin Basic Protein as would be typical in measles viral infections of the CNS. Other viruses are known to do this as well, eg, Japanese Encephalitis, but we have no evidence or history for any of the other candidate infections. So, we see:

- Presence of the virus in 82% of regressed/bowel patients compared to a very low number of controls. This represents a documented unique inflammatory bowel disease in ASD children. (Uhlmann 2002 & Wakefield 2000)
- Autoimmunity in the presence of the virus (gut and brain – published by Singh, et al & the group at Royal Free). Present in cases not controls.
- Antibodies to the virus in the CSF - not seen in controls. (Singh & Bradstreet 2002).
- Virus genome (F gene) in the CSF - no controls yet. High correlation between MV in blood of cases (currently 100% of those with suspected brain MV). (Presented here).
- Frequent seizures (typical of MV in the CNS, but not specific for any one virus).
- Depletion of cysteine and sulfur (Waring et al 2000), consistent with a persistent viral infection, not specific for MV - also seen in HIV patients.

- Resultant 500% higher levels of Hg in cases over controls. (ICDRC – IOM presentation of Bradstreet, 2001).

4) In that last several months, a senior investigator for the Committee and I have conducted an informal poll of numerous pediatricians, neurologists and immunologist. We have provided laboratory results and histories. Then we asked them to give us their best diagnosis for the cases. Every physician was unanimous that the findings represent measles infection in the brain. They differed somewhat on the nature of the infection, but only over whether it represented acute infection or subacute sclerosising panencephalitis (SSPE).

5) In Scott's situation, Dr John Menkes the esteemed professor of pediatric neurology and author of the foundational textbook *Child Neurology,* agrees that the findings can only be interpreted to represent measles infection in the brain. He refers to this as atypical SSPE, since it does not appear to be causing the typical findings in SSPE. That may be somewhat confusing terminology. I would prefer to call it ***autoimmune encephalopathy with measles virus persistence.*** I believe Professor Menkes and I remain in complete agreement about the disease process, and as with this entire problem – just need to come to terms about the nomenclature. Menkes would no doubt have an identical interpretation of my son's case.

So who and what are we to believe. Everyone agrees the epidemiology is not precise enough to detect rare events. But are these two boys rare? Certainly the data of Singh , Uhlmann, Wakefield, Bradstreet and others represent a much larger population than just these two cases. Several hundred children have been studied and published in the various papers. While we need controls and confirmation for this most recent piece of the puzzle (viral genome in the CSF), I think we should be more than concerned about the findings. My impression from carefully examining and investigating 1500 cases of autism is that these boys are not isolated cases. I am terribly concerned they may well represent the majority of cases of regressed autistic encephalopathy children.

Therefore, we as a society need ask and answer some important questions:

- **What if Wakefield, Singh, Bradstreet and Menkes are right about these data.?**
- **What then?**
- **Have we traded acute measles and occasional SSPE from the wild disease for a 1 in 80 risk for boys to develop this new form of measles disease?**
- **Can that be a justifiable risk/benefit ratio?**
- **Do we have safer vaccines?**
- **If so, why aren't we using them? It appears Dr Bellanti at Georgetown does have a safer measles vaccine that he cannot get licensed.**
- **What has held up the approval of that measles vaccine?**
- **If we do not have safer vaccines, why don't we?**
- **How are we going to treat these two boys, or the potential hundreds of thousands like them?**
- **Why doesn't the epidemiology agree with the biology? Have we asked the right questions in the way the epidemiology studies were constructed? Did they use reliable methods and databases?**
- **Is it only a reaction to MMR or are many things capable of triggering the brain autoimmunity and gut disorders we are seeing?**
- **Do, as I suspect thimerosal, aluminum, and the various vaccine antigens prime the immune system to respond abnormally to live virus injections?**
- **We suspended live polio vaccines for early life because of 9 cases of polio in susceptible individuals. How many persistent measles autistic-like encephalopathies will it take to stop using MMR and find a safer vaccine alternative? Are 10 enough? 100? 1000? Or do we need hundred's of thousands of cases?**
- **In a similar light, how many inflammatory bowel diseases must it cause?**

- **Should live viruses be injected, thereby violating the normal immune mechanisms, or should they always be provided through natural route of infection means?**
- **Should live viruses ever be used, or is this playing with immunological and virological fire?**

I am only asking these questions to stimulate reasoned medical debate and investigation. I am convinced about the nature of our present autism epidemic, but I also recognize changing the course for vaccine policy is like changing the course for a large ocean going vessel, hopefully it will not be like the *Titanic*. Presently, my partner, Dr Kartzinel and I have a waiting list to get on our waiting list. We hear from parents daily with newly diagnosed children. I will once again remind the Congress of the words of our Surgeon General:

"Growing numbers of children are suffering needlessly because their emotional, behavioral, and developmental needs are not being met by those very institutions, which were explicitly created to take care of them." Surgeon General Sachet (http://www.surgeongeneral.gov/cmh/childreport.htm)

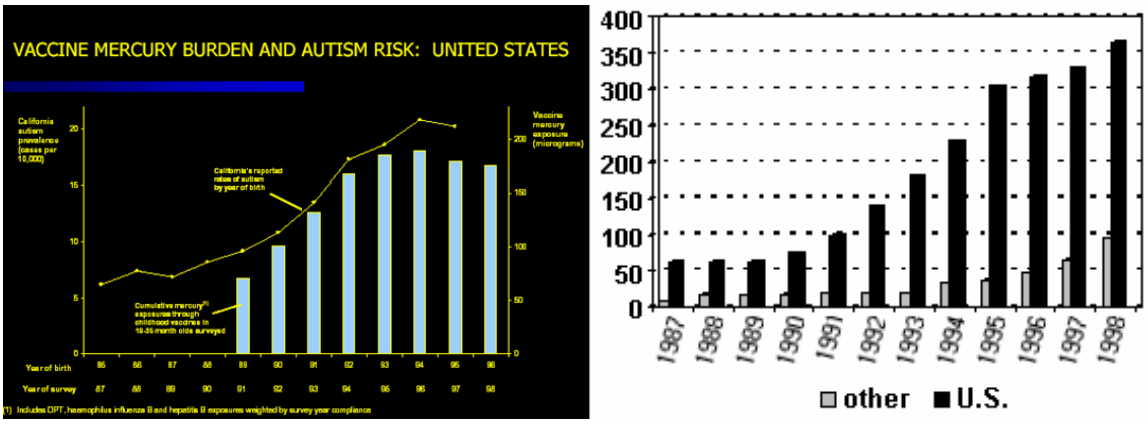
Epidemiology (with the known flaws in the published studies) has failed to find an association, although the data sources used are suspect. The most difficult piece of data is the continued rise in prevalence despite flat uptake of the vaccine. Our explanation of that observation has to do with other priming events for MMR which are not constant over the time in question.

So how is it the IOM and the various expert bodies looking at this data come away saying there is NO evidence of a link between MMR and ASD? They ignore all of these molecular viral data and immunological findings and rely slavishly upon rather poor epidemiology. Is ASD multi-factorial? It must be - humans are far to complex to be

reduced to simplistic & mechanistic processes when brain development is involved. Is it just MMR? I doubt it for most cases.

The better question is this: is it ultimately MMR? I certainly see the evidence for that - again - in most cases. This, I believe is the result of numerous antecedent priming events – including the right genetic predisposition to certain immunological events – such as autoimmunity.

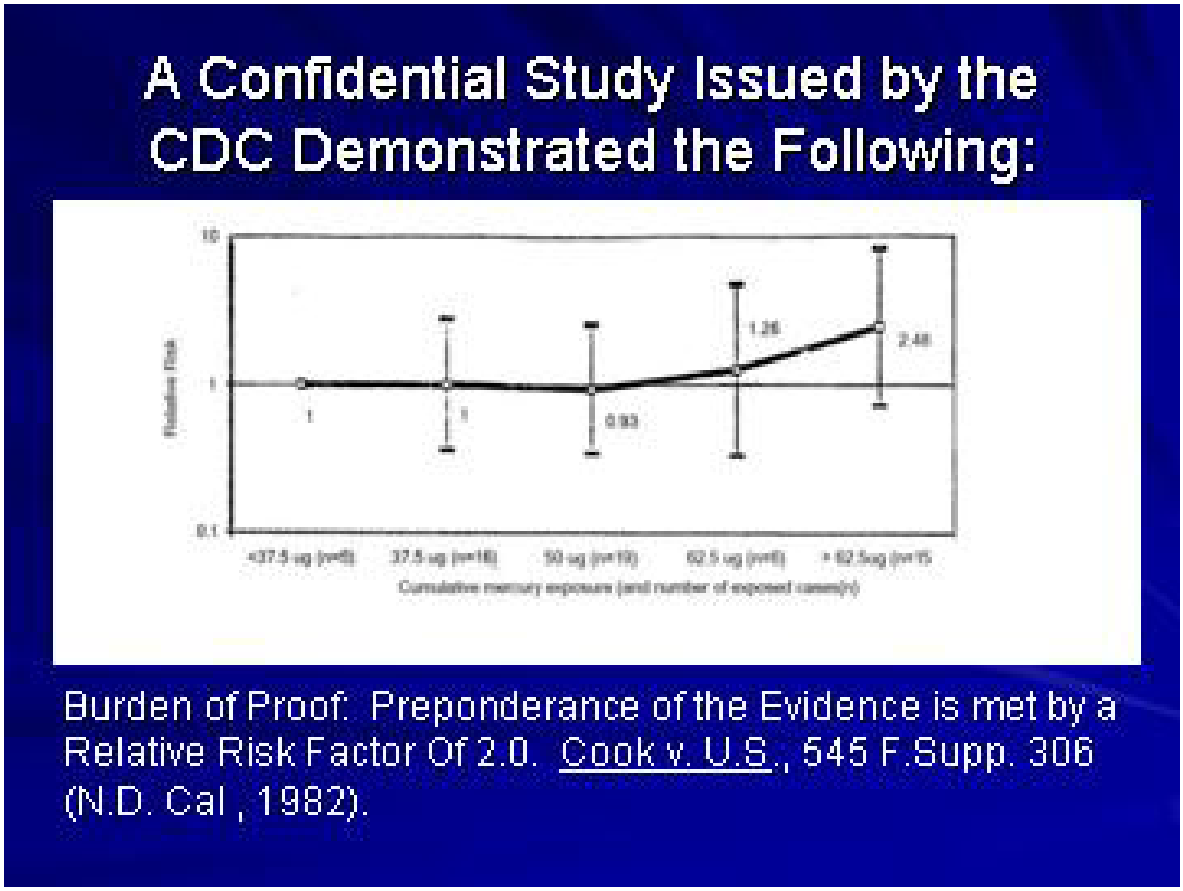
There is even more reason for concern. The CDC was willing to present data that Thimerosal vaccines were associated with a statistically significant increased risk of Attention Deficit Hyperactivity Disorder. Below I compare the chart prepared and presented to the IOM by Mark Blaxil and the US data on stimulant use for ADHD. It seems obvious there is a significant relationship between the two – both start to rise abruptly around 1990.



Cumulative Dose of Mercury from Vaccine Burden and Autism rates in California on the left side and United Nations Data Methylphenidate Consumption (Defined Daily Doses in millions) on the right side.

Source:http://www.ahcpr.gov/clinic/epcsums/adhdsutr.htm

Add these data to the findings of the original CDC study on thimerosal and autism risk. See slide below:



This moves my discussion into: Legal Concerns Congress Must Keep in Mind

Last year I predicted that if there was not immediate action to address the growing understanding of thimerosal toxicity and MMR, that this country would face potentially catastrophic legal consequences. I made several recommendations, some of which are echoed in the proposed amendments to the Vaccine Injury Compensation Fund, (VIC

Fund) supported by both sides of this Committee. But sending parents to the "Fund" for compensation for their child's needs is literal purgatory.

As we search for truth in determining the safety, or lack thereof, for the many vaccine components, we must keep in perspective there exists two separate systems for determining medical truth in this country: 1) the realm scientific purity which is largely impossible to obtain in pediatric research and practice, and 2) the legal or tort system.

The requirements to satisfy the Institute of Medicine, from my personal dealings with them, might be greater than 90% certainty prior to affirming any casual relationship to vaccine components. But the tort system defines things differently. While it will not be my place to offer legal definitions to the committee, it suffices to say that our courts and/or special masters will soon answer the question regarding vaccine linkage to autism.

Having been an expert at several causation hearings for vaccine "Fund" cases, it is clear this system will in no way benefit the children affected by ASD, even if the table were amended. The program is broken beyond repair and the use of the Justice Department to try cases is unwise. They - by the nature of legal practices - take an adversarial position against the parents – whom are already suffer through tremendous financial and emotion hardships. Presently 85% of our ASD families have ended in divorce. Clearly then, a non-adversarial system must be created, or again the thousands of children enrolled in class- action or private suits against vaccine manufacturers and distributors will quickly become hundred's of thousands.

A primer on causality from Attorneys Kenneth S Lewis and Ann-Louise Kleper: *"To the lawyer, "cause" includes not only that which precipitates, initiates or produces, but also that which accelerates, aggravates or worsens some medical condition. In other words, the definition embraces elements which bring about symptoms, disability, damages or death sooner than would have ordinarily been expected in the normal course of the underlying disease. Such a concept is inherently foreign to scientific thinking,*

which considers the underlying reason for the entire disorder. After considering all of the factors underlying the disorder and their inter-relationships, medicine seeks to ascertain the cause - the single element responsible for the condition of ill-being, the identity of which may be demonstrated clearly and conclusively. ***In law, exclusivity may not be demanded... The evidence necessary to establish causation in medicine must be verifiable by objective diagnostic methods; physicians demand scientific proof. The law is too pressed for time to allow the parties the luxury of such certainty. If disputes are to be resolved and if justice is to be done, a decision cannot be postponed until medical science advances to the point where all questions posed by a particular claim may be unequivocally determined."***

And what ultimately determines "scientific proof" always seems to be debatable amongst the experts themselves. So, while medical types piously discuss the purity of research, the courts grind on. Inaction by Congress and Administrations (current and past) has allowed a tragic epidemic to go unnoticed, except by those directly involved. While there may be some hope based on recent meetings I have had with HHS, government response to the crisis is still painfully slow. As regrettable as our present reality is – it does appear families will be turning to the courts to resolve their grievances in huge numbers. And that will be, to quote the Bard: "A pox on both your Houses."

Regardless of the ultimate legal outcome, everyone will loose something.

- The child with autism will loose irreplaceable time as the cost of required treatment goes unmet by both governmental and insurance providers.
- The vaccine manufacturers will pay vast amounts in legal defense and thereby loose money which might be used to generate safer vaccines. If the courts find against the vaccine industry, the losses could be staggering – beyond any prior tort awards given the nature of ASD and the huge numbers of children affected.
- Parents have already lost their peace of mind regarding public health policy, but the public legal battle will no doubt erode remaining confidence in vaccines even further regardless of the science – doubt will be fostered.

- Society will continue to loose the productive contribution of parents and children consumed by ASD.

Again I ask Congress and the Administration to address the needs for families and the appropriate funding for ASD treatment, therapy and research. In any other clinical setting the information we have gathered on children would be far more than what would be needed to make a diagnosis. Is it enough evidence? Most certainly! Is it proof? Well, I guess that depends on what you call proof. Is it more than the 50% assurance as would be required in a legal setting? Is it 100%? No, but things rarely are in medicine - especially early in the evolution of knowledge on a new finding.

Respectfully Submitted,

James Jeffrey Bradstreet, MD, Fellow, AAFP
Director, Clinical Services
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REFERENCES & ABSTRACTS:

Serological Detection of Measles Virus in Relation to Autoimmunity in Autism

102nd General Meeting of the American Society for Microbiology
May 19-23, 2002, Salt Lake City, Utah, Presentation V-5

V.K. Singh, R.L. Jensen, J. J. Bradstreet
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Abstract: Autoimmunity to brain myelin protein (MBP) secondary to a measles infection may cause autistic regression in some children with this neurodevelopmental disorder. We hypothesized that measles-mumps-rubella (MMR) immunization is a source of measles infection; hence the serological link between MMR and MBP antibodies might exist in autistic children. To test the hypothesis, we conducted a serological study of MBP, MMR and neuron-axon filament protein (NAFP) in serum and cerebral spinal fluid (CSF) of autistic children. Antibodies were assayed by immunoblotting with MBP, NAFP and MMR as antigens. We found that a significant number of autistic children had antibodies to MBP (up to 88% positive) and antibodies to MMR (up to 65% positive), but not to NAFP. Normal children did not harbor these antibodies. Moreover, the analysis of paired samples (serum and CSF) from 7 autistic children also revealed a high degree of serological association between MMR and MBP: 50% of CSF had MMR antibodies, 86% of CSF had MBP antibodies, 75% of sera had MMR antibodies and 100% of sera had MBP antibodies. Therefore, as indicated by paired analysis of serum and CSF samples, there is a strong correlation between MMR antibodies and MBP autoantibodies in autism. By using monoclonal antibodies, we characterized that the MMR antibodies are due to the measles subunit, but not due to mumps or rubella subunits, of the polyvalent vaccine. Furthermore, the MMR and MBP antibodies are not cross-reactive because the pre-incubation of MBP with MMR did not block the binding of MBP antibodies. In light of the new evidence presented here, we suggest that the MMR vaccine in some cases of autism might cause autoimmunity and it might do so by bringing on an atypical measles infection that does not produce a typical measles rash but manifests neurological symptoms upon immunization.

Note: The MMR antibody has been previously reported to be the hemaggluttin protein of the vaccine measles virus (MV-HA)**. "Immunoblotting analysis showed the presence of an unusual MMR antibody in 60% (75 of 125) of autistic children, but <u>none</u> of the 92 normal children had this antibody. Moreover, by using MMR blots and monoclonal antibodies, we found that the specific increase of MV antibodies or "MMR" antibodies was related to**

measles hemagglutinin antigen (MV-HA)," (Singh, VK. Abnormal Measles Serology and Autoimmunity in Autistic Children, *Journal of Allergy Clin Immunol* 109 (1):S232, Jan. 2002.) It is confirmed here (in an additional population) that this antibody is not typically produced during normal immune response to the vaccine.

Molecular Psychiatry (2002) ●●, ●●●–●●●

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism

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We have reported lymphocytic colitis in children with regressive autism, with epithelial damage prominent. We now compare duodenal biopsies in 25 children with regressive autism to 11 with coeliac disease, five with cerebral palsy and mental retardation and 18 histologically normal controls. Immunohistochemistry was performed for lymphocyte and epithelial lineage and functional markers. We determined the density of intraepithelial and lamina propria lymphocyte populations, and studied mucosal immunoglobulin and complement C1q localisation. Standard histopathology showed increased enterocyte and Paneth cell numbers in the autistic children. Immunohistochemistry demonstrated increased lymphocyte infiltration in both epithelium and lamina propria with upregulated crypt cell proliferation, compared to normal and cerebral palsy controls. Intraepithelial lymphocytes and lamina propria plasma cells were lower than in coeliac disease, but lamina propria T cell populations were higher and crypt proliferation similar. Most strikingly, IgG deposition was seen on the basolateral epithelial surface in 23/25 autistic children, co-localising with complement C1q. This was not seen in the other conditions. These findings demonstrate a novel form of enteropathy in autistic children, in which increases in mucosal lymphocyte density and crypt cell proliferation occur with epithelial IgG deposition. The features are suggestive of an autoimmune lesion.
Molecular Psychiatry (2002) ●, 000–000. DOI: 10.1038/sj/mp/4001077

Keywords: autism; small intestine; inflammation; lymphocytes; immunoglobulins; autoimmunity; complement

Mol Psychiatry 2002;7(4):375-82

ORIGINAL ARTICLE

Potential viral pathogenic mechanism for new variant inflammatory bowel disease

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J Clin Pathol: Mol Pathol 2002;**55**:0–6

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Accepted for publication 8 November 2001

Aims: A new form of inflammatory bowel disease (ileocolonic lymphonodular hyperplasia) has been described in a cohort of children with developmental disorder. This study investigates the presence of persistent measles virus infection in the intestinal tissue of these patients (new variant inflammatory bowel disease) and a series of controls by molecular analysis.

Methods: Formalin fixed, paraffin wax embedded and fresh frozen biopsies from the terminal ileum were examined from affected children and histological normal controls. The measles virus Fusion (F) and Haemagglutinin (H) genes were detected by TaqMan reverse transcription polymerase chain reaction (RT-PCR) and the Nucleocapsid (N) gene by RT in situ PCR hybridisation. Localisation of the mRNA signal was performed using a specific follicular dendritic cell antibody.

Results: Seventy five of 91 patients with a histologically confirmed diagnosis of ileal lymphonodular hyperplasia and enterocolitis were positive for measles virus in their intestinal tissue compared with five of 70 control patients. Measles virus was identified within the follicular dendritic cells and some lymphocytes in foci of reactive follicular hyperplasia. The copy number of measles virus ranged from one to 300 000 copies/ng total RNA.

Conclusions: The data confirm an association between the presence of measles virus and gut pathology in children with developmental disorder.

Aliment Pharmacol Ther 2002; 16: 1–12.

Entero-colonic encephalopathy, autism and opioid receptor ligands: an hypothesis

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Accepted for publication ■ ■ 2001

MMR vaccine

New evidence for a viral pathogenic mechanism for new variant inflammatory bowel disease and development disorder?

A Morris, D Aldulaimi

See article by Uhlmann *et al* page XXX

We are all aware of the public unease about a potential link between vaccination with the triple vaccine MMR (mumps, measles, and rubella) and autism or bowel inflammatory conditions, with some hundreds of parents of afflicted children undertaking legal action against the manufacturers. There is no space to go into detail of the controversy over the link here (search the web using keywords "measles, MMR, vaccination, autism")—suffice it to say that reliable epidemiologists are content that there is no significant association between MMR and either autism or bowel inflammatory conditions. However, epidemiology is a pretty blunt tool and the studies done do not rule out the possibility that there may be "at risk" groups where a real link between MMR and autism/bowel inflammatory conditions exists.

"Epidemiology is a pretty blunt tool and the studies done do not rule out the possibility that there may be at risk groups where a real link between MMR and autism/bowel inflammatory conditions exists"

In 1998, Wakefield and colleagues reported colitis and ileal lymphoid nodular hyperplasia in children with developmental disorders such as autism, and suggested a possible link between MMR vaccination and a chronic enterocolitis associated with neuropsychiatric dysfunction in these children.[1] In 2000, a further study by the same group supported the association of developmental disorders with a distinct form of inflammatory bowel disease—new variant inflammatory bowel disease.[2]

In this present paper,[3] the authors report the association of this condition with the persistence of at least fragments of the measles virus genome within the follicular dendritic cells and lymphocytes of areas of lymphoid nodular hyperplasia. The technique used (reverse transcriptase polymerase chain reaction) could not indicate whether whole virus was present, or whether it was replicating, but for the moment we can go along with the notion that the virus is persisting in some form in these patients.

The interpretation of this finding is difficult. It would be entirely wrong to jump to the conclusion that the measles component of MMR "causes" the colitis or the developmental disorder in these particular (or any other) children. Causation is rarely simple and never pure: most if not all diseases are multifactorial in nature, and the data here could equally well be interpreted as indicating that the colitis or the developmental disorder "cause" the persistence of the measles. The measles virus persistence could reflect the inability of patients with a developmental disorder to clear the virus. The enterocolitis may cause failure of viral clearance. And in no way can the data presented here be used to support the generalisation that MMR causes all autism and/or inflammatory diseases of the bowel.

There is evidence that developmental disorders are associated with a functional disturbance of the brain–gut axis. Neurogenerative disorders such as Parkinson's disease and functional bowel diseases, such as the irritable bowel syndrome, are associated with abdominal pain, bloating, and diarrhoea. Functional magnetic imaging has demonstrated striking differences in cortical activation following colonic distension in patients with irritable bowel syndrome compared with normal controls, suggesting that a disturbance in perception in the absence of obvious pathological changes may lead to abdominal pain, bloating, and diarrhoea. Thus, the symptoms present in the patients with developmental disorders may result from pathological modulation of the functional interface between the immune and sensory motor systems of the gut. Hence, disturbance of the brain–gut axis might lead to alterations in local neurotransmitters and mediators of inflammation—and so failure to clear virus infections efficiently.

"There is evidence that developmental disorders are associated with a functional disturbance of the brain–gut axis"

The data presented here are unquestionably interesting but beg a string of further questions: Is replicating whole virus present? Is it identical to the vaccine strain? Are other viruses—mumps or rubella—present? What about the nature of immunity to measles and other pathogens in these children? These questions come immediately to mind. Doubtless the present (and other) authors are pursuing these (and many other) questions: we look forward to answers.

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Neuro-immunopathogenesis in Autism

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Infection of Human B Lymphocytes with MMR Vaccine Induces IgE Class Switching

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Circulating immunoglobulin E (IgE) is one of the characteristics of human allergic diseases including allergic asthma. We recently showed that infection of human B cells with rhinovirus or measles virus could lead to the initial steps of IgE class switching. Since many viral vaccines are live viruses, we speculated that live virus vaccines may also induce IgE class switching in human B cells. To examine this possibility, we selected the commonly used live attenuated measles mumps rubella (MMR) vaccine. Here, we show that infection of a human IgM$^+$ B cell line with MMR resulted in the expression of germline ε transcript. In addition, infection of freshly prepared human PBLs with this vaccine resulted in the expression of mature IgE mRNA transcript. Our data suggest that a potential side effect of vaccination with live attenuated viruses may be an increase in the expression of IgE.

Key Words: vaccine; IgE; asthma; allergy.

INTRODUCTION

A key component in allergic reactions is increased levels of circulating immunoglobulin E (IgE) (1, 2). Mature resting B cells express IgM and subsequently can further differentiate to undergo immunoglobulin class switching and secrete immunoglobulins with IgG, IgA, or IgE isotopes. Upon interaction with allergens and crosslinking of specific IgE molecules bound to the high-affinity surface receptors, mast cells and basophils release several mediators. The release mediators such as histamine and leukotrienes are responsible for many clinical manifestations of allergic responses (3–5).

The incidence rate of allergic reactions such as asthma has increased in the past 20 years. However, the reason for this increase is not yet clear. Since genetic background of the population has not changed in a significant way, several environmental factors have been suggested. One proposed factor is the improvements in home construction leading to an increase in the indoor humidity and temperature resulting in an increase in house dust mite and cockroach allergens (6, 7). Bacterial products such as CpG nucleotides are thought to down-regulate allergic differentiation; therefore, others have suggested that an increase in antibiotic usage and a subsequent reduction in bacterial infections has created an environment that may favor allergic conditions (8, 9). Moreover, a decrease in childhood outdoor activities and an increase in sedentary life styles have been suggested to be contributory to the increase in asthma incidence (6, 10).

Another intriguing mechanism for the increasing incidence of allergic reactions is viral exposure during childhood. Our recent reports have demonstrated that viral infections can modulate IgE class switching in human B cells (11, 12). The induction of class switching was subsequent to activation of protein kinase R (PKR), NF-κB, and STAT-6. This is consistent with targeted disruption studies in mice demonstrating that deletion of the gene encoding the p50 subunit of the NF-κB complex and STAT-6 resulted in a reduction in the level of serum IgE, suggesting a critical role for these nuclear factors in IgE class switching (13–16).

To induce protective immunity against pathogenic viral diseases, live attenuated viruses are used in several childhood vaccines such as polio, MMR (mumps, measles, rubella), and varicella. Based on the previously published data and our recent reports we addressed the hypothesis that live viral vaccines can induce IgE class switching. In this report we provide evidence that infection with MMR vaccine can induce IgE class switching in a human B cell line and freshly prepared peripheral blood lymphocytes (PBLs).

MATERIALS AND METHODS

Cell culture conditions, vaccine infections, and reagents. The human Burkitt's lymphoma Ramos B cell line at 1×10^5–1×10^6 cells/ml was grown in RPMI 1640 supplemented with 10% fetal calf serum, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate

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1521-6616/01 $35.00

"Vaccination provides great protection against the mortality and morbidity associated with many childhood diseases and should not be discouraged, **but it is possible that a side effect of viral vaccination constitutes an increase in the incidence of IgE-mediated disorders. A better understanding of the mechanism underlying this event may yield improved vaccines in the future." Imani and Kehoe, page 360.**

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Sonoda S, Nakayama T.
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A total of 342 samples of peripheral blood mononuclear cells (PBMC) were obtained from 145 healthy individuals, which we examined for the presence of measles virus genome RNA by reverse transcription-polymerase chain reaction (RT-PCR), to identify whether asymptomatic infection of measles virus has occurred in healthy children. Measles virus genome was detected in 11 (23.4%) of 47 nonimmunized individuals; all positives for RT-PCR were infants who experienced measles exposure. No genome was detected in those without measles exposure. In 83 individuals immunized with measles vaccine, the vaccine strain genome was detected in 10 (71.4%) of 14 recipients whose PBMC were obtained within 2 months of vaccination. Measles wild-type genome was detected in 36 (46.2%) of 78 individuals, 40 (25.2%) of 159 samples, who had been immunized more than 2 months before. The wild-type measles genome was also detected in 6 (46.2%) of 13 individuals who had been infected with measles in the distant past. The measles PCR-positive rate was not related to the period since immunization or natural infection. Sequence analysis of PCR products demonstrated they were all in the same cluster of D5 lineage, which was the circulating strain during the study period. We obtained 13 samples of nasopharyngeal secretion (NPS) simultaneously from individuals whose PBMC were positive for measles PCR but did not detect virus genome. Measles genome was, however, detected from NPS in cases of acute infection. We conclude that asymptomatic measles infection is common but would rarely become a source of transmission because of negative PCR in NPS. Copyright 2001 Wiley-Liss, Inc.

J Med Virol 1998 Oct;56(2):174-7
Partial amplification of the measles virus nucleocapsid gene from stored sera and cerebrospinal fluids for molecular epidemiological studies.
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The analysis of stored sera for retrospective molecular epidemiological studies provides a powerful tool to investigate strain variation in measles viruses that had circulated up to 20 years ago. For this purpose, a

rapid and simple method for extraction of RNA from stored sera and cerebrospinal fluids (CSF) was developed. When used on sera and CSFs that have been frozen for as long as 20 years, this method proved to be more efficient than established techniques. The extracted RNA was reverse transcribed into cDNA by using random hexamer primers. The PCR amplification of the 3' terminus of the nucleocapsid gene (N) was divided into two overlapping fragments of 375 and 384 bp length, covering the entire region of interest. This region is thought to have the highest variability within the MV genome and has previously been shown to be suitable for strain characterization. The resulting PCR fragments were sequenced manually by using standard methods without the need of further clean-up steps.

Brain Dev 1996 May-Jun;18(3):220-3
A case of intractable epilepsy positive for the detection of measles virus genome in the cerebrospinal fluid and peripheral mononuclear cells using reverse transcriptase-polymerase chain reaction.
Kawashima H, Miyajima T, Mori T, Yuan L, Ogihara M, Kinoue K, Takekuma K, Hoshika A.
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We report a rare case of intractable frontal lobe epilepsy with mental deterioration, in which the measles virus gene was detected from the cerebrospinal fluid (CSF) and peripheral mononuclear cells (PBMC) obtained 9 years after the first epileptic episode using reverse transcriptase-polymerase chain reaction (RT-PCR). The patient had been immunized with an attenuated measles vaccine and had no history of clinically apparent acute measles infection. However the analysis of the sequence of the PCR product from CSF showed the circulating wild strain genotype at the time when the patient complained of his first epileptic episode.

Psychiatry Clin Neurosci 1995 Jun;49(3):S294-5
A case of intractable epilepsy with mental deterioration: detection of measles virus genome in cerebrospinal fluid and peripheral mononuclear cells using reverse transcriptase-polymerase chain reaction.
Miyajima T, Kawashima H, Hoshika A, Ogihara M, Yamada N, Wang CY, Kinoue K, Takekuma K.

Virus Res 1995 Jan;35(1):1-16
Detection of measles virus genome directly from clinical samples by reverse transcriptase-polymerase chain reaction and genetic variability.
Nakayama T, Mori T, Yamaguchi S, Sonoda S, Asamura S, Yamashita R, Takeuchi Y, Urano T.

A simple and sensitive method for the detection of measles virus genome was developed, amplifying the regions encoding the nucleocapsid (N) protein and hemagglutinin (H) protein of measles virus by reverse transcriptase-polymerase chain reaction (RT-PCR). We examined a variety of measles patients: 28 patients with natural infection, 4 with measles encephalitis and 1 with subacute sclerosing panencephalitis (SSPE). In 28 patients with natural measles infection a single step PCR amplifying the N region resulted in a high detection rate for all plasma samples (28/28) within 3 days of the onset of rash and 80% (20/25) even on day 7 of the onset of rash and later. Within 3 days of the onset of rash, 24/25 (96.0%) of nasopharyngeal secretions (NPS) and 27/28 (96.4%) of peripheral blood mononuclear cells (PBMC) were positive for the N region PCR and the positivity rate of PCR decreased in NPS and PBMC after 7 days of the rash. In acute measles infection, measles genome was detected in all cell fractions, CD4, CD8, B cells, and monocytes/macrophages by the H gene nested PCR. Measles genome was also detected from cerebrospinal fluids (CSF) in patients with measles encephalitis, SSPE, and acute measles by the H gene nested PCR. PCR products of the N and H regions were sequenced and we confirmed the presence of measles genome. Based on the sequence data, chronological sequence differences were observed over the past 10 years. The sequences obtained from the SSPE patient were closely related to those of the wild viruses that were circulating at the time when the patient initially acquired measles. RT-PCR for NPS, PBMC, CSF, and plasma provides a useful method for the diagnosis of measles and molecular epidemiological study in addition to virus isolation.

Ann Neurol 1994 Jul;36(1):103-8

Subacute sclerosing panencephalitis in an infant: diagnostic role of viral genome analysis.

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Subacute sclerosing panencephalitis (SSPE) is related to "defective" measles virus or vaccination, though an association with parainfluenza viruses has been reported. SSPE is characterized by a slow, erratic course and elevated cerebrospinal fluid measles titers. An immunocompetent, vaccinated infant, with onset of symptoms in parainfluenza virus season and a catastrophic course is described. Cerebrospinal fluid titers were negative, but postmortem brain had typical SSPE lesions. Patient brain-derived RNA, subjected to reverse transcription followed by polymerase chain reaction yielded polymerase chain reaction products with measles virus but not parainfluenza virus genes. The sequenced fragment revealed multiple mutations, typical for SSPE. SSPE can thus present in infants, with short latency and no cerebrospinal fluid antibodies. Viral genomic analysis may be diagnostic, permitting early therapy.

J Med Virol 1990 Apr;30(4):237-44

Detection of measles virus genomic sequences in SSPE brain tissue by the polymerase chain reaction.

Godec MS, Asher DM, Swoveland PT, Eldadah ZA, Feinstone SM, Goldfarb LG, Gibbs CJ Jr, Gajdusek DC.

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The polymerase chain reaction (PCR) was modified to detect RNA genomic sequences by generating cDNA copies of these sequences as a preliminary step. Oligonucleotide primer pairs complementary to sequences in each of the five major structural protein genes of the measles virus (nucleocapsid protein, phosphoprotein, matrix protein, fusion protein, and hemagglutinin protein) were synthesized. PCR products were tentatively identified by visualization of bands of the appropriate size by ethidium bromide staining after gel electrophoresis, and identity was confirmed by subsequent restriction enzyme cleavage of the products at predetermined sites to yield fragments of predicted size. This method successfully amplified 400-500 base regions from each of these five genes in RNA extracts of wild measles virus cultured in Vero cells and in RNA extracted from most of the SSPE brain tissues tested, but not in RNA from any control brain tissues. Measles virus genome was detected in SSPE brain tissues stored frozen for as long as 27 years and formalin-fixed paraffin-embedded subacute sclerosing panencephalitis (SSPE) brain tissues as old as 9 years. This method provides a simple, rapid and highly sensitive means of detecting and identifying sequences of RNA genomes by PCR. The success of this method in detecting measles virus in SSPE brain tissue suggests that PCR is appropriate to investigate the possible presence of RNA viruses in other neurological disorders of unknown etiology.

Infection of human B lymphocytes with MMR vaccine induces IgE class switching.

Clin Immunol 2001 Sep;100(3):355-61

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Circulating immunoglobulin E (IgE) is one of the characteristics of human allergic diseases including allergic asthma. We recently showed that infection of human B cells with rhinovirus or measles virus could lead to the initial steps of IgE class switching. Since many viral vaccines are live viruses, we speculated that live virus vaccines may also induce IgE class switching in human B cells. To examine this possibility, we selected the commonly used live attenuated measles mumps rubella (MMR) vaccine. Here, we show

that infection of a human IgM(+) B cell line with MMR resulted in the expression of germline epsilon transcript. In addition, infection of freshly prepared human PBLs with this vaccine resulted in the expression of mature IgE mRNA transcript. **Our data suggest that a potential side effect of vaccination with live attenuated viruses may be an increase in the expression of IgE.**