# Enteroviral meningoencephalitis as a complication of X-linked hyper IgM syndrome

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We describe 5 children from 2 families with mutations in the CD40 ligand (CD40L) gene leading to absent expression of CD40L on activated CD4 cells. All subjects presented with interstitial pneumonia with low serum IgG and normal serum IgM. One child had normal and one child had elevated serum IgA. Four had confirmed *Pneumocystis carinii* pneumonia. In spite of intravenous immunoglobulin treatment yielding therapeutic serum immunoglobulin levels, 3 children had enteroviral encephalitis. When assessed by flow cytometry, the 3 surviving affected male children had absent CD40L expression on activated $CD4^+$ T cells. The affected children from both families were shown to have the same single nucleotide insertion (codon 131) resulting in frameshift and early termination within exon 4 (extracellular domain). This observation demonstrates that persistent enteroviral infection is not only observed in X-linked agammaglobulinemia but may also occur in patients with X-linked hyper IgM syndrome. (J Pediatr 1999;134:584-8)

X-linked hyper IgM syndrome is a rare cause of immunodeficiency characterized by normal or elevated levels of IgM and low levels of IgG, IgA, and IgE. Clinical manifestations include neutropenia, cholangitis, tumors, and frequent infections with bacteria, *Pneumocystis carinii*, *Candida* spp, and cryptosporidium.[1] XHIM is caused by mutations of the gene encoding CD40 ligand.[2] This protein is expressed on the surface of activated $CD4^+$ T cells and interacts with CD40, a receptor constitutively expressed on B cells.[3] This interaction leads to B-cell activation and switching of immunoglobulin classes.[4] Failure to express functional CD40L interferes with normal B-cell function, resulting in abnormal antibody responses to T-cell–dependent antigens, failure to switch from IgM to other immunoglobulin isotypes, lack of immunologic memory, and inability to form germinal centers in lymph nodes.[5]

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We describe 5 children from 2 families with XHIM, as documented by the absence of CD40L expression on activated $CD4^+$ T cells and identical mutations within exon 4 of the CD40L gene. All 5 patients presented with interstitial pneumonia and 3 had progressive echovirus infections, a complication previously reported in only one patient.[1]

| | |
|---|---|
| CD40L | CD40 ligand |
| CSF | Cerebrospinal fluid |
| IVIG | Intravenous immunoglobulin |
| PCP | *Pneumocystis carinii* pneumonia |
| PCR | Polymerase chain reaction |
| PMA | Phorbol 12-myristate 13-acetate |
| TMP/SMZ | Trimethoprim sulfamethoxazole |
| WBC | White blood cell |
| XHIM | X-linked hyper IgM |

## Case Reports

The 5 affected male children were from 2 African American families and presented to the same institution over a 5-year period. The families lived in the same community but had no known relationship to one another. The family pedigrees are shown in the Figure. Clinical and immunologic findings are summarized in the Table.

### *Family 1*

Patient III:3 (F1PIII:3) presented at 8 months of age with interstitial pneumonia, oral thrush, and mild developmental delay. Open lung biopsy confirmed the presence of *Pneumocystis*

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*carinii*. He was treated with intravenous immunoglobulin (400 mg/kg) and intravenous trimethoprim/sulfamethoxazole. His condition was stable with monthly IVIG infusions and TMP/SMZ for *P carinii* pneumonia prophylaxis until 30 months of age when, over a 3-week period, he had marked loss of developmental milestones including inability to run and loss of vocabulary. Examination revealed increase in deep-tendon reflexes and bilateral ankle clonus. Cerebrospinal fluid white blood cell count was 16 cells/mm$^3$ with 100% mononuclear cells, and CSF protein and glucose concentrations were normal. CSF viral culture was negative, but echovirus 14 was isolated from a nasopharyngeal swab. CSF polymerase chain reaction was positive for enterovirus. He began receiving IVIG, 1 g/kg/d, but continued to deteriorate rapidly. An Omaya reservoir was placed for intraventricular administration of IgG. He died 1 year later from central hypoventilation caused by profound central nervous system dysfunction. Echovirus 14 was isolated from respiratory tract specimens after 2 months of therapy; however, subsequent CSF PCRs for enterovirus were negative.

Patient III:4 (F1PIII:4) is the younger brother of F1PIII:3. He was well until 11 months of age when interstitial pneumonia and hypoxia developed. Studies for PCP were not performed, but he received 7 days of TMP/SMZ therapy. At that time, CSF showed a WBC count of 14 cells/mm$^3$ with 100% mononuclear cells. Protein and glucose concentrations were normal. No bacteria or viruses were identified from CSF cultures. He was discharged with a prescription for IVIG, 400 mg/kg per month, and TMP/SMZ for PCP prophylaxis. Two months later, he was noted to be neutropenic (absolute neutrophil count of 140 cells/mm$^3$), a finding that persisted for 1 year but resolved with granulocyte colony-stimulating factor treatment. Because the neutropenia developed during treatment with TMP/SMZ, he was given intravenous pentamidine therapy on a monthly basis. He had persistent mild developmental delay of unknown etiology, although he continued to achieve new developmental milestones. When he was 19 months of age and his brother was found to have enteroviral encephalitis, he had CSF studies performed, for which the results were normal; enteroviral PCR was normal. At 21 months he presented with a 2-week history of irritability and the abrupt loss of milestones. CSF at this time was negative by culture for viruses and bacteria, but PCR was positive for enterovirus. A nasopharyngeal culture was positive for echovirus 22. He began receiving IVIG, 1.5 g/kg per week, and neurologic function rapidly returned to baseline. Three months later, CSF was negative for enterovirus by culture and PCR. He continued to receive IVIG every 1 to 2 weeks and intravenous pentamidine every month without interruption. He was doing well until 3 years of age when he presented with pneumonia confirmed to be caused by *P carinii* by a direct fluorescent antibody stain of endotracheal secretions. He was successfully treated with daily intravenous pentamidine. He is now 9 years old and has severe developmental delay and spastic quadriparesis.

### *Family 2*

Patient III:5 (F2PIII:5) presented at age 7 months with PCP requiring mechanical ventilation. After recovery he was treated with monthly IVIG infusions and TMP/SMZ. Over the next 2 years, nasopharyngeal cultures were intermittently positive for different enteroviruses. At 30 months of age he had nasopharyngeal cultures that were persistently (>1 month) positive for echovirus 11 and mild developmental delay. His CSF at that time showed a WBC count of 9 cells/mm$^3$ with 100% mononuclear cells. The CSF cultures were negative for viruses and bacteria, but PCR was positive for enterovirus. He had clinical improvement after his IVIG infusions were given more often (ie, weekly). A subsequent CSF sample showed an increase in WBC count to 50 cells/mm$^3$, but PCR was negative for enterovirus. He is now 9 years of age and has remained clinically stable with no new infections. He continues to have mild developmental delay but normal gross motor skills.

Patient III:6 (F2PIII:6) is the younger brother of F2PIII:5. He presented with bilateral interstitial pneumonia at age 5 months. Studies for PCP were not performed. The pneumonia resolved after therapy with broad-spectrum antibiotics and TMP/SMZ. He has been treated with monthly infusions of IVIG and oral TMP/SMZ prophylaxis and has had no significant infections. At the age of 18 months, he had CSF studies done because of persistence of echovirus 11 in his upper airway. CSF showed a WBC count of 9 cells/mm$^3$ with 90% mononuclear cells and normal concentrations of glucose and protein. CSF PCR was negative for enterovirus. He is now 7 years old and has normal growth and development.

Patient III:1 (F2PIII:1), a first cousin to patients F2III:5 and F2III:6, presented at 7 months of age with PCP. The mothers are half sisters, with a common father. Despite aggressive therapy with TMP/SMZ, IVIG, and corticosteroids, he died on the 13th hospital day.

## METHODS

### *Viral Culture and PCR*

Enteroviral cultures were performed by using standard methodologies. Positive isolates were presumptively identified by cytopathic effects in Rhesus monkey kidney cells and identified by neutralization assays at the New York State Department of Health Laboratory.

Enterovirus detection by reverse-transcription PCR was performed as previously reported.[6,7] Oligonucleotide primers and probe were chosen from the highly conserved 5′ end of the enteroviral genome. The amplifica-

***Table.*** Immunologic evaluation at time of presentation and subsequent clinical and virologic findings

| Patient | Months at presentation (death) | IgG (mg/dL) | IgM (mg/dL) | IgA (mg/dL) | Lymphocyte count (cells/μL) | CD4 (%) | CD8 (%) | CD19 (%) | Lymphocyte proliferation assay | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Mitogen | Antigens |
| F1 PIII:3 | 8 (42) | 80 | 72 | 57 | 7344 | 51 | 19 | 20 | Normal | Normal |
| F1 PIII:4 | 11 (alive) | 15 | 20 | 0 | 9000 | 41 | 20 | 38 | Normal | Normal |
| F2PIII:5 | 7 (alive) | <40 | 52 | 0 | 2940 | 43 | 10 | 45 | Normal | Normal |
| F2PIII:6 | 5 (alive) | 62 | 40 | 7 | 3430 | 27 | 16 | 51 | Normal | ND |
| F2PIII:I | 7 (7) | 52 | 51 | 107 | 4960 | 21 | 9 | 69 | ND | ND |

*ND,* Not done.

tion product was detected by gel electrophoresis and confirmed by Southern blot hybridization.

### *CD40L Expression*

CD40L expression was measured by flow cytometry on positively selected $CD4^+$ T cells both at rest and after stimulation with phorbol 12-myristate 13-acetate (10 ng/mL) (Sigma Chemical Co, St Louis, Mo) and ionomycin (400 ng/mL) (Calbiochem-Novabiochem Corp, LaJolla Calif). Purified $CD4^+$ T cells were isolated from peripheral blood mononuclear cells by immunomagnetic separation (Dynabeads M-450 CD4; Dynal, Oslow, Norway). After 1 mL of $5 \times 10^5$ $CD4^+$ T cells/mL was treated with phosphate-buffered saline or PMA/ionomycin, 1 μg of appropriate monoclonal antibody was added and incubated for 18 hours at 37°C in an atmosphere of 5% $CO_2$. Antibodies included mouse anti-human CD40L (Genzyme, Cambridge, Mass) and mouse anti-human CD69 (Becton Dickinson, San Jose, Calif). Cells were washed and incubated with goat anti-mouse fluorescein isothiocyanate for 30 minutes at 37°C. Flow cytometric analysis was performed with scatter gates set on the lymphocyte fraction. Laser excitation was at 488 nm.

### *DNA Analysis*

The mutation of the CD40L gene was identified by direct sequencing of the reverse-transcription PCR product obtained from activated peripheral blood mononuclear cells as described previously.[8] The polymorphism of $(CA)_n$ repeat in the 3′ non-coding region of the CD40L gene and of $(AGAT)_n$ repeat of the HPRT gene was determined according to previously described methods.[9,10]

## RESULTS

### *CD40L Expression*

Expression of the cell activation marker, CD69, was identical in patients and healthy control subjects (mean, 98.0% ± 1.6% cells stain positive; range, 92.6% to 99.8%), indicating that cells could be activated appropriately. In contrast, CD40L expression was markedly diminished in the affected children with an average of 1.2% ± 0.5% of purified $CD4^+$ patient cells staining with CD40L monoclonal antibody after PMA/ionomycin stimulation. In control subjects (healthy adults and children with hypogammaglobulinemia), a mean of 82.5% ± 8.4% (range, 69.9% to 93.6%) cells were positive for CD40L.

### *Mutation Analysis*

The 3 affected children available for study (one from family 1 and two from family 2) were found to have the same point mutation in the CD40L gene, an A insertion in exon 4 between nt C411 and nt G413 (CAGC→CAAGC) (Figure, *C*). This mutation resulted in frameshift, substitution of the following 2 amino acids, and then termination, causing truncation of the extracellular domain. Evaluation of polymorphic (CA) repeats in the 3′ untranslated region of the CD40L gene and of the $(AGAT)_n$ repeat of the HPRT genes suggests that the X chromosomes with the mutated CD40L gene are identical in the 2 families.

## DISCUSSION

Chronic enteroviral meningoencephalitis is known to occur in patients with IgG deficiency, including those with common variable immunodeficiency and Bruton's X-linked agammaglobulinemia.[11] Meningitis, encephalitis, and encephalopathy were recently reported as complications in 12% of subjects with XHIM, although echovirus was identified as the etiologic agent in only 1 of the 7 subjects with neurologic symptoms.[1] Three of the children in this report had enteroviral encephalitis in spite of acceptable trough serum levels of IgG. The enteroviral infection was unlikely present before the initiation of IVIG therapy because the enterovirus serotype isolated from the children at the time of the clinical meningoencephalitis was not detected before the time of central nervous system symptoms and one child had a negative CSF PCR per-

| | Enteroviral studies | |
|---|---|---|
| PCP | PCR (CSF) | Culture (respiratory) |
| Confirmed | + | Echo 14 |
| Confirmed | + | Echo 22 |
| Confirmed | + | Echo 11 |
| Suspected | – | Echo 11 |
| Confirmed | ND | ND |

formed 2 months before acute enteroviral encephalitis developed (F1PIII:4).

The reason for the frequent occurrence of invasive enteroviral disease in these 2 families is uncertain. It is unlikely that this unique mutation makes this group unusually susceptible to enteroviral disease. Trough levels of serum IgG were maintained at >400 mg/dL above baseline in all children before the development of meningitis, and all doses of IVIG were given under direct medical supervision. It is not known whether the lots of IVIG used had significant titers against the children's specific isolates of enterovirus.

Enteroviral PCR for diagnosis proved useful because none of these children grew enterovirus from CSF, although all had persistently positive respiratory cultures and 2 of the children responded clinically, with neurologic/developmental improvement, to increased doses of IVIG. PCR has previously been shown to be more sensitive than culture in diagnosing enteroviral infections in patients with meningoencephalitis and immunodeficiency.[12] The prior and ongoing use of IVIG has modified the clinical presentation of enteroviral encephalitis in patients with low serum IgG concentrations. Subtle abnormalities such as developmental arrest or regression are not uncommon.[13]

PCP has been reported to occur in over 35% of children with XHIM.[1]

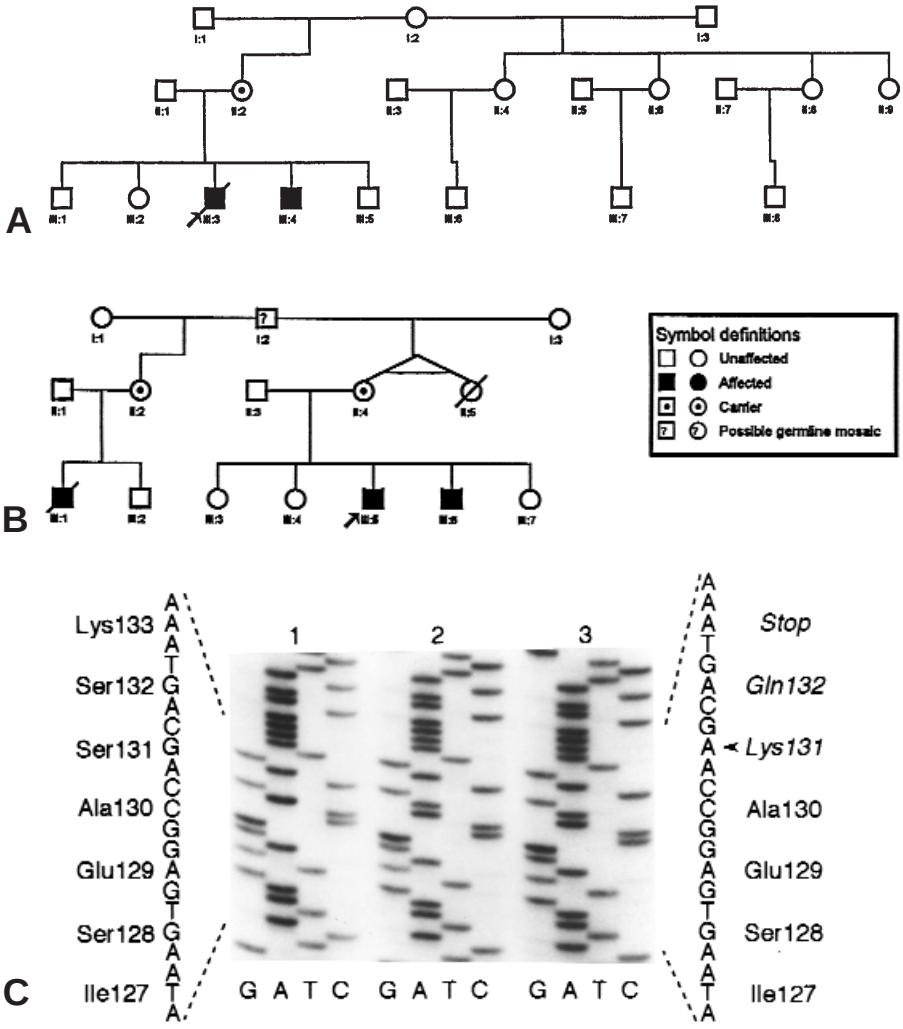


***Figure.*** Pedigrees for relevant family members in the 2 families. Probands are designated with an *arrow*. **A,** Family 1. **B,** Family 2. **C,** Sequence analysis of a region of CD40L cDNA (exon 4, codons 127-133) generated from 2 subjects showing an A insertion in codon 131 (between NT C411 and G413). The lanes identified with 1 represent the control sequence analysis, whereas 2 and 3 are from subjects F1PIII:4 and F2PIII:5, respectively.

Therefore all such children should receive PCP prophylaxis. One child in this study had PCP despite high-dose IVIG therapy and monthly infusions of intravenous pentamidine. TMP/SMZ is considered the drug of choice for the prevention of PCP in high-risk children. Other agents that have been used include aerosolized and intravenous pentamidine and oral dapsone, but failures have been documented with all regimens.[14]

None of the children in these 2 families had elevated serum IgM levels: normal and low serum IgM levels were seen. Normal IgM levels at the time of presentation have been reported for >50% of boys with XHIM.[1] In this regard the syndrome appellation is a misnomer; many patients with CD40L deficiency have normal serum levels of IgM, whereas some patients with elevated serum levels of IgM have normal CD40L.[15] In this cohort one child had normal serum IgA concentration on presentation, and one child had elevated IgA concentration. The only clinical finding on presentation that distinguished these children from those with common variable immunodeficiency was the presence of PCP. Testing for CD40L gene mutations and/or protein

expression should be considered for all boys with significant hypogammaglobulinemia.

The specific mutation identified in these patients has not been previously reported.[16,17] The evaluation of the polymorphic repeats within the CD40L and HPRT genes suggests that the defective gene is from a single source. Because the 2 obligate carrier mothers from family 2 share a common father, it is possible that he is the source of the original mutation. To date, neither the common father nor the mothers of the obligate carriers have agreed to be tested.

The majority of the children in this report had severe disease and poor outcome. Therefore interventions such as bone marrow transplantation or gene therapy should be evaluated as potential treatments for this condition. Successful allogeneic bone marrow transplantation has been reported in 2 unrelated children with XHIM syndrome.[18,19] Bone marrow transplantation was considered for some of the children in this report; however, it was believed that the risk associated with iatrogenic immunosuppression in a child with chronic enteroviral infection was excessive.

In summary, enteroviral meningoencephalitis can occur in children with XHIM in spite of regular administration of IVIG, with resulting therapeutic immunoglobulin levels. Children with this syndrome who have new central nervous system symptoms, including loss of developmental milestones, should have CSF obtained for culture and PCR testing for enterovirus as part of their evaluation.

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