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Detection and Sequencing of Measles Virus from Peripheral Mononuclear Cells from Patients with Inflammatory Bowel Disease and Autism

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It has been reported that measles virus may be present in the intestine of patients with Crohn's disease. Additionally, a new syndrome has been reported in children with autism who exhibited developmental regression and gastrointestinal symptoms (autistic enterocolitis), in some cases soon after MMR vaccine. It is not known whether the virus, if confirmed to be present in these patients, derives from either wild strains or vaccine strains. In order to characterize the strains that may be present, we have carried out the detection of measles genomic RNA in peripheral mononuclear cells (PBMC) in eight patients with Crohn's disease, three patients with ulcerative colitis, and nine children with autistic enterocolitis. As controls, we examined healthy children and patients with SSPE, SLE, HIV-1 (a total of eight cases). RNA was purified from PBMC by Ficoll-paque, followed by reverse transcription using AMV; cDNAs were subjected to nested PCR for detection of specific regions of the hemagglutinin (H) and fusion (F) gene regions. Positive samples were sequenced directly, in nucleotides 8393–8676 (H region) or 5325–5465 (from noncoding F to coding F region). One of eight patients with Crohn disease, one of three patients with ulcerative colitis, and three of nine children with autism, were positive. Controls were all negative. The sequences obtained from the patients with Crohn's disease shared the characteristics with wild-strain virus. The sequences obtained from the patients with ulcerative colitis and children with autism were consistent with being vaccine strains. The results were concordant with the exposure history of the patients. Persistence of measles virus was confirmed in PBMC in some patients with chronic intestinal inflammation. KEY WORDS:

KEY WORDS: measles; inflammatory bowel disease; pervasive developmental disorder.

It has recently been reported that measles virus may be detectable in the intestine in patients with Crohn's disease (1, 2), and a relationship between atypical measles virus infection and later inflammatory bowel disease, including ulcerative colitis, has been proposed. Additionally, a new syndrome has been reported in children with developmental regression progressing to autism, chronic intestinal inflammation, and immunodeficiency (3). Behav-

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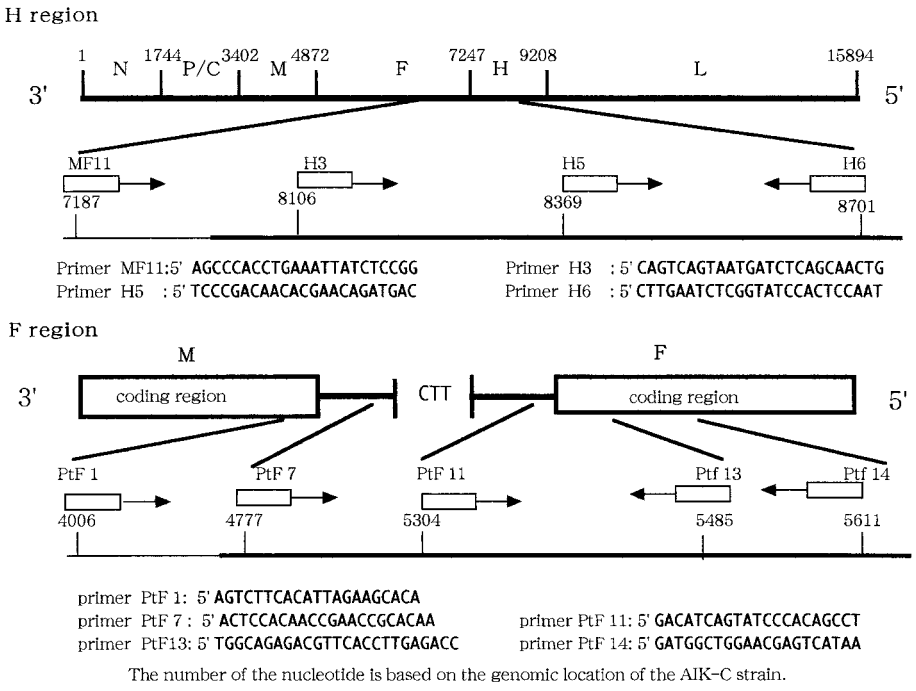

Fig 1. Design of primers for RT-PCR.

TABLE 1. SEQUENCE DATA FOR POSITIVE SAMPLES OBTAINED FROM IBD PATIENTS

	8393	8401	8411	8421	8431	8441	8451	8461
MCS*	AAGTTGCG	AATGGAGACA	TGCTTCCAGC	AGGCGTGTAA	GGGTAAAATC	CAAGCACTCT	GCGAGAATCC	CGAGTGGGCA
Crohn's disease	--------	----------	----------	----------	----------	----------	----------	----------
UC	--------	----------	--------A-	----------	----------	----------	----------	----------
Autistic child	--------	----------	----------	----------	----------	----------	----------	----------
Autistic child	--------	----------	----------	----------	----------	------A---	----------	----------
Autistic child	--------	----------	--------A-	----------	----------	----------	----------	----------
	8471	8481	8491	8501	8511	8521	8531	8541
MCS	CCATTGAAGG	ATAACAGGAT	TCCTTCATAC	GGGGTCTTGT	CTGTTGATCT	GAGTCTGACA	GTTGAGCTTA	AAATCAAAAT
Crohn's disease	----------	----------	-T--------	----------	----------	----------	----------	----------
UC	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	---G------	----------	----------	----------	----------	----------
Autistic child	----------	-A--------	-----T----	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	---CC-----	---------C	----------	----------
	8551	8561	8571	8581	8591	8601	8611	8621
MCS	TGCTTCGGGA	TTCGGGCCAT	TGATCACACA	CGGTTCAGGG	ATGGACCTAT	ACAAATCCAA	CCACAACAAT	GTGTATTGGC
Crohn's disease	----------	----------	----------	----------	----------	----------	----------	----------
UC	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	----------	----------	----------	-----C----
Autistic child	----------	----------	----------	---------T	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	----------	----------	----------	----------
	8631	8641	8651	8661	8671			
MCS	TGACTATCCC	GCCAATGAAG	AACCTAGCCT	TAGGTGTAAT	CAACAC			
Crohn's disease	----------	----------	----------	----------	------			
UC	----------	----------	----------	----------	------			
Autistic child	----------	--------G-	----------	----------	------			
Autistic child	----------	----------	----------	----------	------			
Autistic child	----------	----------	----------	----------	------			

* Majority consequences sequence of the measles virus H region antigenomic using all wild-type and vaccine strain sequences from GenEMBL on June 1, 1994.

TABLE 2. COMPARISON OF SEQUENCE DATA FOR POSITIVE SAMPLES OBTAINED FROM IBD PATIENTS WITH VACCINE AND SPORADIC STRAINS

	8393	8401	8411	8421	8431	8441	8451	8461
MCS*	AAGTTGCG	AATGGAGACA	TGCTTCCAGC	AGGCGTGTAA	GGGTAAAATC	CAAGCACTCT	GCGAGAATCC	CGAGTGGGCA
Vaccine (Schwarz)	--------	----------	--------A-	----------	----------	----------	----------	----------
Sporadic wild strain								
Edmonston (1960)	--------	----------	----------	----------	----------	----------	----------	----------
–1985	--------	----------	----------	----------	----------	----------	----------	----------
1985–89	--------	----------	----------	----------	----------	----------	----------	----------
1990–	--------	----------	----------	----------	----------	----------	----------	----------
Crohn's disease	--------	----------	----------	----------	----------	----------	----------	----------
UC	--------	--------A-	----------	----------	----------	----------	----------	----------
Autistic child	--------	----------	----------	----------	----------	----------	----------	----------
Autistic child	--------	----------	----------	----------	----------	----------	----------	----------
Autistic child	--------	----------	--------A-	----------	----------	----------	----------	----------

	8471	8481	8491	8501	8511	8521	8531	8541
MCS	CCATTGAAGG	ATAACAGGAT	TCCTTCATAC	GGGGTCTTGT	CTGTTGATCT	GAGTCTGACA	GTTGAGCTTA	AAATCAAAAT
Vaccine (Schwarz)	----------	----------	----------	----------	----------	----------	----------	----------
Sporadic wild strain								
Edmonston (1960)	----------	----------	----------	----------	----------	----------	----------	----------
–1985	----------	----------	------G---	----------	----------	----------	----------	-------G--
1985–89	----------	----------	----------	----------	-----A----	----------	----------	----------
1990–	---C------	-C--------	----------	----------	-----A----	----------	--------C-	----------
Crohn's disease	----------	----------	----------	----------	----------	----------	----------	----------
UC	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	---G------	----------	----------	----------	----------	----------
Autistic child	----------	-A--------	-----T----	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	---CC-----	---------C	----------	----------

	8551	8561	8571	8581	8591	8601	8611	8621
MCS	TGCTTCGGGA	TTCGGGCCAT	TGATCACACA	CGGTTCAGGG	ATGGACCTAT	ACAAATCCAA	CCACAACAAT	GTGTATTGGC
Vaccine (Schwarz)	----------	----------	----------	----------	----------	----------	----------	----------
Sporadic wild strain								
Edmonston (1960)	----------	----------	----------	----------	----------	----------	----------	----------
–1985	----------	----------	----------	----------	----------	----------	----------	----------
1985–89	------A---	----------	----------	----------	----------	-----A----	----------	----------
1990–	------A---	----------	----------	----------	----------	----------	----------	----------
Crohn's disease	----------	----------	----------	----------	----------	----------	----------	----------
UC	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	---------T	----------	----------	----------	-----C----
Autistic child	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	----------	----------	----------	----------

	8631	8641	8651	8661	8671
MCS	TGACTATCCC	GCCAATGAAG	AACCTAGCCT	TAGGTGTAAT	CAACAC
Vaccine (Schwarz)	----------	----------	----------	----------	------
Sporadic wild strain	----------	A---------	----------	----------	------
–1985	----------	----------	----------	----------	------
1985–89	----------	----------	----------	----------	------
1990–	----------	----------	----------	----------	------
Crohn's disease	----------	----------	----------	----------	------
UC	----------	----------	----------	----------	------
Autistic child	----------	--------G-	----------	----------	------
Autistic child	----------	----------	----------	----------	------
Autistic child	----------	----------	----------	----------	------

* Majority consequences sequence of the measles virus H region antigenomic using all wild-type and vaccine strain sequences from GenEMBL on June 1, 1994.

ioral symptoms developed soon after MMR vaccination in the majority of cases.

Previous RT-PCR-based studies have failed to identify measles virus RNA in the intestinal tissues of patients with Crohn's disease (4–6). We have reported the detection of measles virus genomic RNA in the peripheral blood of both adults and children with autoimmune hepatitis, and we have presented the nucleotide differences in the H region between vaccine and wild strains (7). Applying the same technology, in this study we have investigated the sequences of the H and F regions of the measles genome in the peripheral mononuclear cells of patients with either Crohn's disease, ulcerative colitis, or autistic enterocolitis.

MATERIALS AND METHODS

Of the patients with typical inflammatory bowel disease (IBD), there were eight cases of Crohn's disease (three Japanese and five UK cases) (18–34 years of age), and three cases with ulcerative colitis (one Japanese and two UK

TABLE 3. COMPARISON OF SEQUENCE DATA OBTAINED FROM PATIENT WITH CROHN'S DISEASE IN F REGION WITH VACCINE STRAINS AND SPORADIC WILD STRAINS

	5325 5383
AIK-C	CTCCAAGTCCCCCGGTCTCCTCCTCTTCTCGAAGGGACCAAAAGATCAATCCACCACACCCGACGACACT
FF-8	----------
TO-97	----------
Edmonston	----------
Edmonston wild	----------
1984	----------T---------G----------T----------
1988	----------A----------T----------
1988	----------A----------T----------
1989	----------T----------
1992	----------T----------
1993	----------T----------
1993	----------T----------
1994	----------T----------
1994	----------T----------
Crohn's disease	----------T----------

	5465
AIK-C	CAACTCCCCACCCCTAAAGGAGACACCGGGAATCCCAGAATCAAGACTCATCCAATGTCCATCATGGGTCT
FF-8	----------
TO-97	----------T----------G----------
Edmonston	----------G----------G----------
Edmonston wild	----------T----------
1984	----------A--T----------T----C-T----------
1988	---T-T--------C----------T--------G----------
1988	---T----------C----------G----------
1989	---T-T--------C----------G----------
1992	--G----------C----------C----------A---------G----------
1993	--G----------C----------A---C----------G----------
1993	--G----------C----------C----------C-------G----------
1993	--G----------T------C-----A----------G----------
1994	--G----------C----------G----------
1994	--G----------C----------C----------T--------G----------
Crohn's disease	--G----------C----------G----------

cases) (15–31 years of age). Nine children with autistic enterocolitis—proven by ileocolonoscopy and histology—were all UK cases (3–10 years of age). These children have been reported on elsewhere. All had ileal lymphoid nodular hyperplasia and nonspecific colitis [neither Crohn's disease nor ulcerative colitis (3)]. Twenty-two healthy controls, two HIV-infected patients, and four patients with SLE were also studied.

The methods of reverse transcriptase–polymerase chain reaction (RT-PCR) and direct sequencing was performed as follows: Peripheral blood mononuclear cells (PBMC) obtained from patients and healthy controls were purified by using Ficoll-paque (Pharmacia Biotech) gradient centrifugation from 5 ml of heparinized blood. After washing, RNA was extracted by the acid guanidinium–thiocyanate–phenol–chloroform method, as reported by Chomczynski et al (8). RT-PCR and direct sequencing was performed in a similar way as reported by Nakayama et al (9). cDNA synthesis for the F region and the H region by AMV reverse transcriptase (Life Science) was done with the use of specific adaptor primers named MF1. The first and second rounds of nested PCR were performed as described previously (9). The design of the oligonucleotide primers is given in Figure 1. All oligonucleotide primers were synthesized with reference to the sequence of the AIKC strain reported by Mori (10). Amplification of cDNA by PCR was undertaken using a Perkin-Elmer DNA Thermal Cycler (Applied Biosystems Japan, Inc., Tokyo, Japan). The amplification products were separated by electrophoresis using NuSeive 3:1 agarose (FMC, Rockland, Maine) and stained with ethidium bromide. To confirm the specificity and to identify the strain characteristics, the fragments were sequenced directly with Taq Dye Primer Cycle Sequencing Kit (Applied Biosystems Japan, Inc., Tokyo, Japan) and analyzed using a model 373A sequencer (Applied Biosystems Foster City, California).

RESULTS

The H gene sequences obtained from peripheral mononuclear cells are shown in Table 1. One of eight patients with Crohn's disease, a 31-year-old Japanese male who was infected with measles virus at the age of 8, was positive. One of three patients with ulcerative colitis, a 24-year old Japanese female vaccinated at the age of 1, was positive. Three of nine patients with autism were positive for measles H gene by RT-PCR. Sequences were obtained from all brain tissue samples from cases of SSPE (Table 1), but not from PBMC from other cases of confirmed SSPE. Samples from healthy controls

TABLE 4. COMPARISON OF SEQUENCE DATA FOR POSITIVE SAMPLES OBTAINED FROM IBD PATIENTS WITH SSPE STRAINS

	8393	8401	8411	8421	8431	8441	8451	8461
MCS*	AAGTTGCG	AATGGAGACA	TGCTTCCAGC	AGGCGTGTAA	GGGTAAAATC	CAAGCACTCT	GCGAGAATCC	CGAGTGGGCA
Crohn's disease	--------	----------	----------	----------	----------	----------	----------	----------
UC	--------	----------	--------A-	----------	----------	----------	----------	----------
Autistic child	--------	----------	----------	----------	----------	----------	----------	----------
Autistic child	--------	----------	----------	----------	----------	------A---	----------	----------
Autistic child	--------	----------	--------A-	----------	----------	----------	----------	----------
SSPE 81 (UK)	--------	----------	----------	----------	----------	----------	----------	----------
SSPE 83 (UK)	--------	----------	----------	----------	----------	----------	----------	----------
SSPE 214 (UK)	--------	----------	----------	----------	----------	----------	----------	----------
SSPE 300 (UK)	--------	----------	----------	----------	----------	----------	----------	----------
SSPE 75 (Japan)	--------	----------	----------	----------	----------	----------	----------	----------
SSPE 92 (Japan)	--------	----------	----------	----------	----------	----------	----------	----------

	8471	8481	8491	8501	8511	8521	8531	8541
MCS	CCATTGAAGG	ATAACAGGAT	TCCTTCATAC	GGGGTCTTGT	CTGTTGATCT	GAGTCTGACA	GTTGAGCTTA	AAATCAAAAT
Crohn's disease	----------	----------	----------	----------	----------	----------	----------	----------
UC	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	---G------	----------	----------	----------	----------	----------
Autistic child	----------	-A--------	-----T----	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	---CC-----	---------C	----------	----------
SSPE 81 (UK)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 83 (UK)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 214 (UK)	----------	----------	---G------	----------	----------	----------	----------	----T-----
SSPE 300 (UK)	----------	-A--------	----------	----------	----------	---------G	----------	----------
SSPE 75 (Japan)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 92 (Japan)	----------	----------	------G---	----------	----------	----------	----------	-------G--

	8551	8561	8571	8581	8591	8601	8611	8621
MCS	TGCTTCGGGA	TTCGGGCCAT	TGATCACACA	CGGTTCAGGG	ATGGACCTAT	ACAAATCCAA	CCACAACAAT	GTGTATTGGC
Crohn's disease	----------	----------	----------	----------	----------	----------	----------	----------
UC	----------	----------	----------	----------	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	----------	----------	----------	-----C----
Autistic child	----------	----------	----------	---------T	----------	----------	----------	----------
Autistic child	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 81 (UK)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 83 (UK)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 214 (UK)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 300 (UK)	----------	----------	----------	---------T	----------	----------	----------	----------
SSPE 75 (Japan)	----------	----------	----------	----------	----------	----------	----------	----------
SSPE 92 (Japan)	----------	----------	----------	----------	----------	----------	----------	-C--------

	8631	8641	8651	8661	8671
MCS	TGACTATCCC	GCCAATGAAG	AACCTAGCCT	TAGGTGTAAT	CAACAC
Crohn's disease	----------	----------	----------	----------	------
UC	----------	----------	----------	----------	------
Autistic child	----------	--------G-	----------	----------	------
Autistic child	----------	----------	----------	----------	------
Autistic child	----------	----------	----------	----------	------
SSPE 81 (UK)	----------	----------	----------	----------	------
SSPE 83 (UK)	----------	----------	----------	----------	------
SSPE 214 (UK)	----------	----------	----------	----------	------
SSPE 300 (UK)	----------	----------	----------	----------	------
SSPE 75 (Japan)	----------	----------	----------	-G--------	------
SSPE 92 (Japan)	----------	----------	----------	----------	------

* Majority consequences sequence of the measles virus H region antigenomic using all wild-type and vaccine strain sequences from GenEMBL on June 1, 1994.

and patients with other diseases were all negative. The sequencing data of the H and F regions are shown in Tables 2 and 3. The sequences of wild strains were obtained from data reported by Nakayama et al (9). The sequences obtained from the patient with Crohn's disease showed the characteristics of wild strains. Sequences obtained from the patients with ulcerative colitis and children with autism, were more consistent with the characteristics of the vaccine strain (Table 1). These results were in agreement with the exposure histories of the patients. According to the sequence data of the F region from the patient with Crohn's disease, the measles virus was classified as a sporadic strain that circulated in Japan after 1985.

Comparison between the sequencing data of the H region with that of SSPE strains is shown in Table 4. There were no common differences within the data

Table 5. Comparison of Sequence Data Obtained from Patient with Crohn's Disease in F Coding Region with Vaccine Strains, Sporadic Wild Strains, and SSPE

	5395	5465
AIK-C	CAACTCCCCACCCCTAAAGGAGACACCGGGAATCCCAGAATCAAGACTCATCCA**ATG**TCCATC**ATG**GGTCT	
FF-8	----------	
TO-97	----------T----------G---------	
Edmonston	----------G----------G---------	
Edmonston wild	----------T----------	
1984	----------A--T----------T----C-T----------	
1988	---T-T--------C----------T--------G----------	
1988	---T----------C----------G----------	
1989	---T-T--------C----------G----------	
1992	--G----------C----------C----------A---------G----------	
1993	--G----------C----------A---C----------G----------	
1993	--G----------C----------C----------C------G----------	
1993	--G----------T------C-----A----------G----------	
1994	--G----------C----------G----------	
1994	--G----------C----------C----------T--------G----------	
Crohn's disease	--G----------C----------**G-**----------	
SSPE 75	----------A--T----------T----------	
SSPE 92	----------	

obtained from the patients with IBD and SSPE. Comparison between the sequence data of the first coding region obtained from IBD with those of SSPE is shown in Table 5. In the first F coding region, there are two ATG codons. We compared the region of the sequence data obtained from the patients with Crohn's disease with those of vaccine, wild, and SSPE strains. The sequence data obtained from the patients with Crohn's disease did not have two ATG codons like the vaccine and SSPE strains.

DISCUSSION

In this study, the persistence of measles virus in peripheral blood was confirmed in some patients with chronic intestinal inflammation. It was confirmed that both vaccine and sporadic virus could be persistent. Previous studies, including those of some of the authors, have failed to detect measles virus in Crohn's disease by RT-PCR (4–6). Most studies have investigated the N gene region of the measles virus. We have also carried out RT-PCR for N gene regions; however, all samples were negative (data not shown). The reason for this is not clear, although it is consistent with the experience of others. It is possible that, as with SSPE virus, the persistent strains may have many, although distinct, mutated nucleotides (11).

We have previously reported the persistence of measles genomic RNA in peripheral mononuclear cells from patients with autoimmune hepatitis and intractable epilepsy (7, 10). Detection of measles virus has also been reported in peripheral mononuclear cells in other autoimmune diseases (13). From our data it is not clear whether the measles virus is a causative agent of IBD. However, the persistence of measles virus *in vitro* was reported to enhance MHC class I expression (14), potentially rendering persistently infected cells targets for autoimmune attacks. In addition, it has been reported that the persistence of measles virus in human cell lines is associated with enhanced production of IL-6 and IFN-β (15).

In this study, we could not detect a specific region that was common to all persistent strains, including SSPE. The F gene region may be important in the process of persistent infection (16). We investigated from the noncoding to the coding region of the F region. In these regions there are two ATG codons in the vaccine strain. Many wild strains have only one ATG codon. These data suggest that the vaccine strain would have a higher replicative activity than those of wild strains. However, the sequence obtained from the patient with Crohn's disease had only one codon. Clearly, the mechanism of persistent measles virus infection in patients with chronic intestinal inflammation is complex and requires further study.

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