Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2922–2926, April 1991
Medical Sciences

Retroviral sequences related to human T-lymphotropic virus type II in patients with chronic fatigue immune dysfunction syndrome

(Epstein–Barr virus syndrome/infectious mononucleosis/myalgic encephalomyelitis/polymerase chain reaction/*in situ* hybridization)

ELAINE DEFREITAS*, BRENDAN HILLIARD, PAUL R. CHENEY†, DAVID S. BELL‡, EDWARD KIGGUNDU, DIANE SANKEY, ZOFIA WROBLEWSKA, MARIA PALLADINO, JOHN P. WOODWARD§, AND HILARY KOPROWSKI

The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104

Contributed by Hilary Koprowski, November 13, 1990

ABSTRACT **Chronic fatigue immune dysfunction syndrome (CFIDS) is a recently recognized illness characterized by debilitating fatigue as well as immunological and neurological abnormalities [Straus, S. E. (1988) *J. Inf. Dis.* 157, 405–412]. Once thought to be caused by Epstein–Barr virus, it is now thought to have a different but unknown etiology. We evaluated 30 adult and pediatric CFIDS patients from six eastern states for the presence of human T-lymphotropic virus (HTLV) types I and II by Western immunoblotting, polymerase chain reaction, and *in situ* hybridization of blood samples. The majority of patients were positive for HTLV antibodies by Western blotting and for HTLV-II *gag* sequences by polymerase chain reaction and *in situ* hybridization. Twenty nonexposure healthy controls were negative in all assays. These data support an association between an HTLV-II-like virus and CFIDS.**

The chronic fatigue immune dysfunction syndrome (CFIDS) is characterized by hematologic, neurologic, and neuromuscular abnormalities (1–5). Once called chronic Epstein–Barr virus syndrome, this disease may be related or identical to myalgic encephalomyelitis, Iceland disease, and epidemic neuromyasthenia (6).

Several immunologic abnormalities have been documented in CFIDS patients (7–10). These abnormal immune patterns may represent primary immunologic defects or appear secondary to chronic infection. Although several viruses have been associated with CFIDS, including herpesviruses (11–13) and enteroviruses (14), the ubiquitous nature of these agents has hampered identification of any one as the etiologic agent. We examined adult and pediatric CFIDS patients for evidence of human retroviruses, human T-lymphotropic virus (HTLV) types I and II. For comparison, we also tested healthy age- and sex-matched controls who were household or casual contacts of these patients and healthy people who had no history of or exposure to CFIDS. All samples were coded and the investigators "blinded."

MATERIALS AND METHODS

Subjects. The first cohort consisted of 10 adults and 2 teenagers from a North Carolina referral practice drawn from six eastern states. All patients met established criteria for CFIDS (1) and 4 of the patients were severely affected and largely homebound. The second cohort consisted of 19 children (<18 years of age) from rural upstate New York, where they were part of a CFIDS epidemic in 1985 (15). Of these 19 children, 13 with mild to moderate symptoms were clustered in four families. Six children were not from family clusters. Three of these had severe symptoms and were homebound for more than 6 months.

Healthy age- and sex-matched exposure controls were recruited from among people in North Carolina and New York who had either sexual or casual contacts with the patients. Blood samples from 20 healthy nonexposure controls were obtained in the Philadelphia area: 10 from adults and 10 from umbilical cords of newborns. All adult donors and mothers of newborns denied symptoms of CFIDS and exposure to CFIDS patients.

All patients and controls were Caucasian and denied histories of blood or gamma globulin transfusions, intravenous drug abuse, and male homosexuality. None of the pediatric patients was known to be sexually active and no evidence of sexual abuse was observed. Sera from all patients and controls were tested by the American Red Cross (Philadelphia) and found negative for human immunodeficiency virus (HIV) antibodies and hepatitis B antigen.

Patients' and exposure controls' heparinized blood samples were coded by the referral clinicians and the investigators were blinded. Each patient and exposure control was bled two or three times over 2 years, depending on patient consent. The nonexposure controls were processed and coded by a single person, who was not involved in the experiments. In all experiments, these control samples were interspersed with samples from patients and exposure controls.

Western Immunoblot. Serum or plasma was tested for antibodies to HTLV-I and -II by Western blotting (16). Density gradient-purified HTLV-I from MT-2 cells was generously provided by V. Kalyanaraman (Advanced Biosciences, Kensington, MD) and used as antigen source in all Western immunoblots. Positive control sera were from HTLV-I-infected tropical spastic paraparesis patients. Negative control sera were from healthy donors from Philadelphia. Strips were scored according to criteria of the American Red Cross: positive samples must contain antibodies to products of at least two viral genes, including *gag*.

DNA Extraction. After proteinase K digestion, DNA was extracted semiautomatically in a nucleic acid extractor (model 340A; Applied Biosystems) (ref. 17, pp. 916–919). DNA from HTLV-I- and -II-infected cell lines was extracted using a second machine. DNA samples were aliquoted, used once for polymerase chain reaction (PCR), and discarded.

PCR. To reduce the risk of laboratory contamination of the PCR, all reactions were carried out in a study-dedicated room. PCR was performed as described (18). Reaction-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HTLV, human T-lymphotropic virus; HIV, human immunodeficiency virus; CFIDS, chronic fatigue immune dysfunction syndrome; PBMC, peripheral blood mononuclear cell.

*To whom reprint requests should be addressed.
†Present address: 10620 Park Road, Suite 234, Charlotte, NC 28210.
‡Present address: 15 Lake Avenue, Lyndonville, NY 14098.
§Present address: 699 Mayfair Street, Philadelphia, PA 19120.

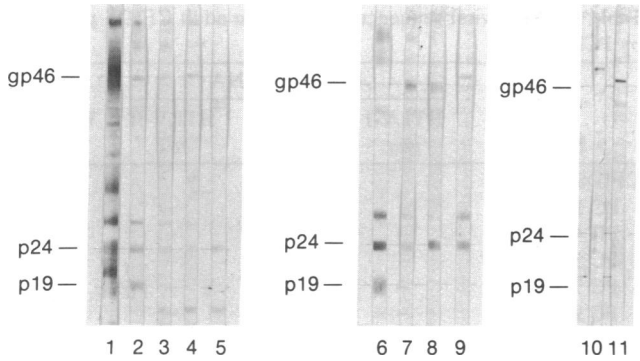

FIG. 1. Western immunoblotting of serum antibodies to HTLV-I in CFIDS patients and controls. Lane 1, typical reactivity of serum from a patient with tropical spastic paraparesis; lanes 2–5, adult cohort; lanes 6–9, pediatric cohort; lanes 10 and 11, typical pattern of nonexposure controls. The molecular mass of viral proteins was determined by comigration of purified proteins (14.3 kDa to 200 kDa).

mixture controls without DNA and with noninfected cellular DNA (U-937 monocytic cells) were included with every PCR.

Oligonucleotides were synthesized and HPLC-purified on a 5–15% acetonitrile gradient in 50 mM triethylamine/acetic acid, pH 7.0, by the Wistar DNA synthesis facility. Amplified DNA was stored at −20°C until use. The template capability of purified DNA was verified by performing a PCR using β-globin primers (19).

Southern Blot Hybridization. Reaction products (25 μl) were electrophoresed in 1.2% agarose gels and capillary-blotted onto Nytran filters (Schleicher & Schuell). The filters were prehybridized as described (ref. 17, pp. 944–955). Oligomer probes were 5′-end-labeled to a specific activity of 6–8 × 10^6 cpm/pmol and hybridized as described (17) at 37°C. The filters were exposed to Kodak XAR film for 5–7 days at −70°C.

***In Situ* Hybridization.** Cells expressing RNA homologous to HTLV-I and HTLV-II were detected by *in situ* hybridization using ^{35}S-labeled RNA antisense probes specific for the 5′ region of both viruses. HTLV-I and HTLV-II *gag* subclones from MT-2 and Mo-T, respectively, were inserted into the pSP64 *Sac* I site and transcribed as reported (20). The RNA transcripts averaged 0.5 kilobase in length and were hybridized at 1–2 × 10^8 dpm/ml at 50°C on 4% paraformaldehyde-fixed activated peripheral blood mononuclear cells (PBMCs). The cells were centrifuged onto glass slides and then autoradiographed for 4–8 days (16).

RESULTS

HTLV Serum Antibodies. We tested sera for evidence of human retroviral infection by Western immunoblotting against purified HTLV-I. By American Red Cross criteria, 50% of adult and 61% of pediatric CFIDS patients were positive, while none of the 20 nonexposure control sera was reactive. Typical patterns of reactivity are shown in Fig. 1. Several CFIDS sera show distinct reactivity to both HTLV *gag* and *env* gene products. CFIDS samples in lanes 2, 3, and 6–9 (Fig. 1) were scored positive, while those in lanes 4 and 5 were negative. The two negative sera (lanes 10 and 11) are representative of the pattern seen with nonexposure controls.

HTLV I gag:	*primers:*	5′CGACCGCCCCGGGGGCTGGCCGCT (841-864 bp)
		5′GGTACTGCAGGAGGTCTTGGAGG (1375-1353 bp)
	probe:	5′GATCCCGTCCCGTCCCGCGCCA (1080-1101 bp)
HTLV II gag:	*primers:*	5′TAAGCTTCAAATCCACGGGCTTTCCCCAACTCC
		5′GAAGCTTTGCGTGGTGGTGGGTTCCACG (1214-1187 bp)
	probe:	5′GTCTCCCCTAGCGCCCCCGCCGCCCC (1080-1105 bp)
HTLV I tax:	*primers:*	5′CAATCACTCATACAACCCCCAA (7575-7586 bp)
		5′TCTGGAAAAGACAGGGTTGGGA (7701-7680 bp)
	probe:	5′TACATGGAACCCACCCTTGGGCAGCA (7652-7677 bp)
HTLV II tax:	*primers:*	5′CGCCTTCCCCGAACCTGGC (7602-7620 bp)
		5′ATAGGGGAGAAGTCCTGTACA (7920-7900 bp)
	probe:	5′ACAGTCATAGTCCTCCCGGAGGACGACC (7819-7846 bp)

FIG. 2. Sequences of primers and probes for HTLV-I and -II *gag* and *tax* (29, 30). bp, Base pairs.

HTLV Proviral Sequences. Since Western blots suggested that a proportion of CFIDS patients may have been exposed to HTLV, we examined their peripheral blood cells for HTLV-I- and -II-specific proviral sequences by PCR. We selected and synthesized a series of oligonucleotides to use as primers and probes specific for the *gag* and *tax* regions of both HTLV-I and -II (Fig. 2).

The results of amplification and Southern blotting for HTLV-I *gag* are shown in Fig. 3. Although a strong signal was observed from MT-2 DNA, all CFIDS patients and controls were negative. Identical results were observed using HTLV-I *tax* primers (data not shown).

In contrast, when the same samples were analyzed for an HTLV-II *gag* region, products of the appropriate size (identical to that in Mo-T) were seen in a majority of adult (Fig. 4*A*) and pediatric (Fig. 4*B*) CFIDS patients. Several exposure controls in both cohorts were also found to contain the same HTLV-II *gag* region as CFIDS patients. These same primers did not amplify any hybridizable product from the blood of 10 healthy infants or 10 healthy adults (Fig. 4 *C* and *D*). (Although shown here in a separate autoradiogram for illustration, these controls were routinely interspersed with patient samples and found negative when assayed.) Despite the lack of amplification of the HTLV-II *gag* region in the nonexposure controls, the DNA from these samples was a competent template for PCR, since a region of β-globin was routinely amplified using specific primers (Fig. 4*E*). All patients were negative for the HTLV-II *tax* amplified product, although this region was detectable in picogram amounts

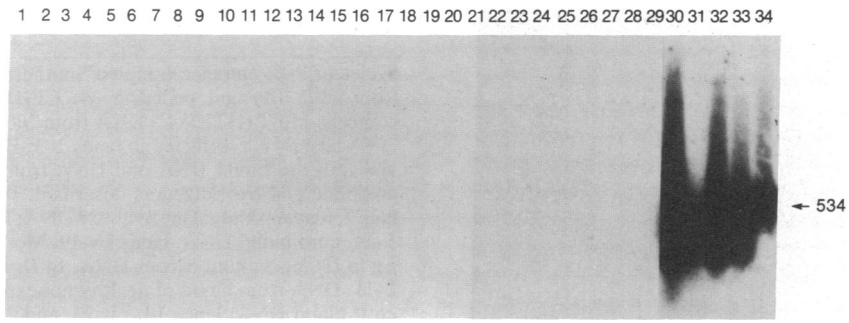

FIG. 3. Amplification and Southern blot hybridization of HTLV-I *gag* in blood from CFIDS patients. After amplification using HTLV-I-specific *gag* primers, DNA was hybridized to an HTLV-I-specific oligonucleotide (see *Materials and Methods*). Lanes 1–12, DNA from adult CFIDS patients; lanes 13–27, pediatric CFIDS DNA; lane 28, U-937 cell DNA; lane 29, no DNA; lanes 30–34, Mo-T (HTLV-II) DNA (200 ng to 20 pg without carrier DNA). Length in base pairs is indicated at right.

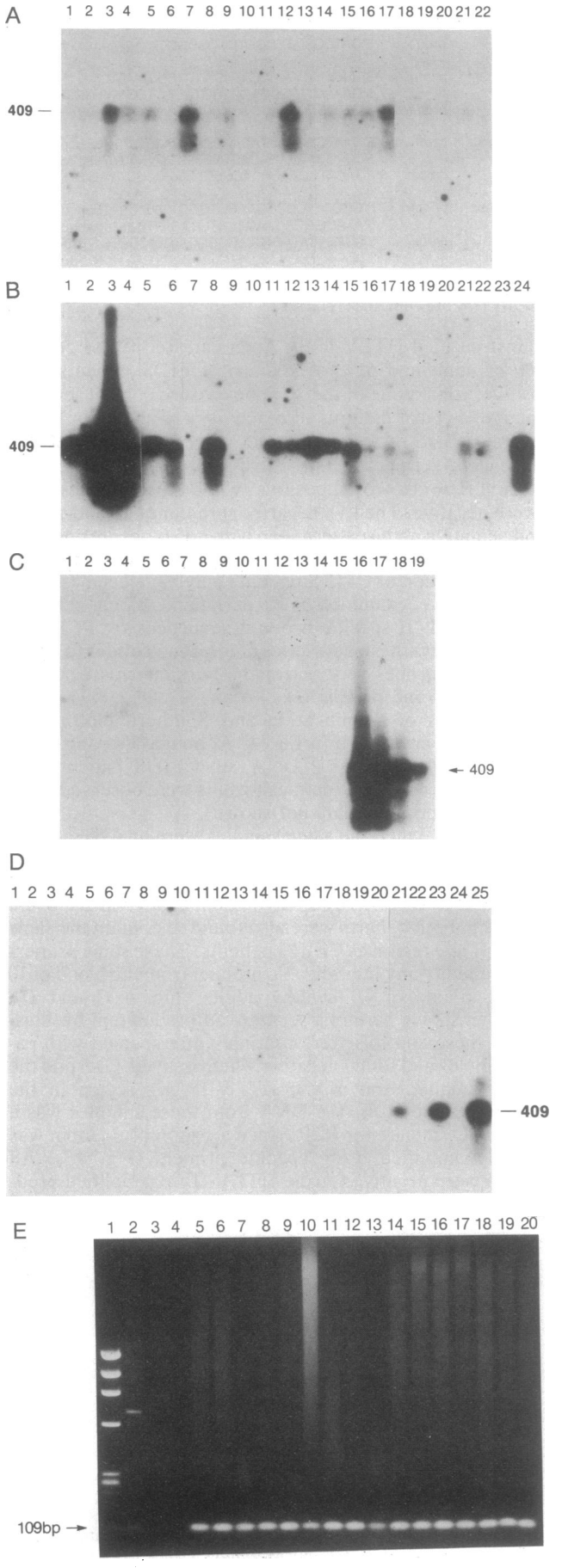

FIG. 4. Detection of HTLV-II *gag* DNA in blood from CFIDS patients, but not from nonexposure controls. (*A* and *B*) After 30 cycles of PCR amplification and Southern blot hybridization, DNA from adult (*A*) and pediatric (*B*) CFIDS patients and exposure controls. Mo-T (HTLV-II) DNA from 200 pg to 2 μg without carrier DNA is shown hybridized in lanes 1–3; HTLV-I DNA in lane 4. (*C* and *D*) Cord blood DNA and DNA from nonexposed adults were amplified and hybridized as described. In *C*: lane 1, φX174 DNA; lane 2, reagents only; lane 3, U-937 DNA; lane 4, buffer control; lanes 5–14, cord blood DNA; lanes 16–19, Mo-T (HTLV-II) DNA from 2 μg to 200 pg without carrier DNA. In *D*: lane 1, φX174 DNA; lanes 2–11, DNA from blood of healthy nonexposure adults; lanes 12–17, cord blood DNA; lanes 19, 21, 23, and 25, Mo-T (HTLV-II) DNA from 2 pg to 2 ng without carrier. (*E*) Amplification of β-globin DNA of adult and pediatric nonexposure controls. Lane 1, φX174 DNA; lanes 2–4, reagent controls; lanes 5–10, DNA from cord blood; lanes 11–20, peripheral blood of adults. Gel was stained with ethidium bromide.

Table 1. Retroviral sequences detected in adult CFIDS patients by PCR

Sample	Retroviral DNA HTLV-I *gag*	Retroviral DNA HTLV-II *gag*	Retroviral DNA HTLV-II *tax*	HTLV Western blot
CFIDS				
PC 1	−	+	−	+
PC 4	−	+	−	−
PC 5	−	+	−	+
PC 7	−	+	−	+
PC 9	−	−	−	+
PC 10	−	+	−	+
PC 11	−	+	−	−
PC 12	−	+	−	−
PC 13	−	−	−	−
PC 14	−	+	−	−
PC 15	−	+	−	−
PC 18	−	+	−	+
Total	0/12	10/12 (83%)	0/12	6/12 (50%)
Controls				
Exposure	0/13	5/13 (38%)	0/13	3/13 (23%)
Nonexposure	0/10	0/10	0/10	0/10
MT-2 (HTLV-I)	+	−	−	
Mo-T (HTLV-II)	−	+	+	

of DNA from Mo-T and was detected in only one exposure control (data not shown). The PCR experiments are summarized in Tables 1 and 2.

Retroviral mRNA Detection. To determine whether the HTLV-II *gag* region amplified by PCR of patients' DNA was part of a functional gene, we evaluated the expression of HTLV mRNA in PBMCs by *in situ* hybridization using *gag* transcripts from both HTLV-I and -II. Fig. 5 shows the typical pattern of reactivity seen with HTLV-II *gag* RNA probe. The analysis of adult samples is summarized in Table 3.

DISCUSSION

We have presented evidence for HTLV-II-like infection of blood cells from CFIDS patients and, to a lesser extent, from some people who closely associate with them. More than 50% of samples from 31 patients contained antibodies to at least two viral gene products by Western blot analysis. Since serologic assays using HTLV-I-derived antigens cannot distinguish between HTLV-I- and -II-specific antibodies, the positive Western blots may be directed toward either virus (21). Because purified HTLV-I was used as the antigen, it is unlikely that the antibodies were detecting cellular antigens shed from the infected MT-2 cells. This is further substantiated by patient reactivity to proteins with the molecular weights reported for HTLV-I and -II antigens. The pronounced reactivity to *gag* (p19 and p24) and *env* (gp45–46) in

Table 2. Retroviral sequences detected in pediatric CFIDS patients by PCR

Sample	Retroviral DNA HTLV-I *gag*	Retroviral DNA HTLV-II *gag*	Retroviral DNA HTLV-II *tax*	HTLV Western blot
CFIDS				
4-4	−	+	−	+
4-4(2)	−	+	−	+
3-4	−	−	−	+
10-4	−	+	−	+
13-16	−	−	−	−
5-16	−	+	−	+
8-16	−	+	−	+
13-2	−	+	−	−
13-2(2)	−	+	−	−
9-2	−	+	−	+
20-2	−	−	−	−
2-2	−	+	−	+
10-18	−	+	−	+
19-4	−	+	−	+
12-2	−	+	−	−
10-2	−	−	−	−
10-10	−	−	−	−
1-16	−	+	−	+
Total	0/18	13/18 (72%)	0/18	11/18 (61%)
Controls				
Exposure	0/7	2/7 (29%)	0/7	3/7 (43%)
Nonexposure	0/10	0/10	0/10	0/10
MT-2 (HTLV-I)	+	−	−	
Mo-T (HTLV-II)	−	+	+	

positive patients suggests exposure to a virus closely related or identical to either HTLV-I or -II.

The frequency of these antibodies in CFIDS patients compared with healthy, noncontact controls suggests exposure/infection with an HTLV-like agent rare in healthy noncontact people. Some adult CFIDS patients in the study were from the southeastern United States, where HTLV-I infections are more frequent, but the reported incidence of HTLV seropositivity in blood donors there is only 0.05% (22). More important, the pediatric patients reside in rural upstate New York, where population studies have shown that the HTLV seropositivity rate approaches zero (22).

Although polyclonal upregulation of antibodies to other viruses has been reported in CFIDS, this mechanism is unlikely with respect to HTLV (23). These antibodies appear to be directed exclusively to viruses to which the majority of the population has been exposed. Polyclonal B-cell activation would not be expected to induce antibodies to a virus the patients had never encountered.

To detect the presence of proviral HTLV in blood cells of CFIDS patients, we amplified specific regions of HTLV-I and HTLV-II by PCR. None of the CFIDS patients' blood samples contained detectable HTLV-I *gag* sequences. In contrast, DNA from at least two separate bleedings was positive for the HTLV-II *gag* subregion in 83% of adult and 72% of pediatric CFIDS patients. None of the HTLV regions examined was amplified from DNA of 20 nonexposure controls. If this *gag* gene is a segment of a complete provirus, the retrovirus is probably not prototypic HTLV-II (24), since we did not amplify an HTLV-II *tax* region in CFIDS patients that is found in Mo-T. The reported sequence variability among HTLV-II isolates, not seen with isolates of HTLV-I, supports this hypothesis (25).

We confirmed these findings on the adult CFIDS samples by demonstrating that 7 out of 10 HTLV-II *gag*-positive samples by PCR had cells expressing HTLV-II *gag* mRNA. Moreover, the detection of viral mRNA in these cells demonstrates that the HTLV-II *gag* is a functional gene. Although the *gag* mRNA-positive cells were rare (10^{-2} to 10^{-4}) as compared with the productively infected T-cell line Mo-T, similar frequencies of PBMCs expressing retroviral mRNA have been reported for HIV-infected individuals (20).

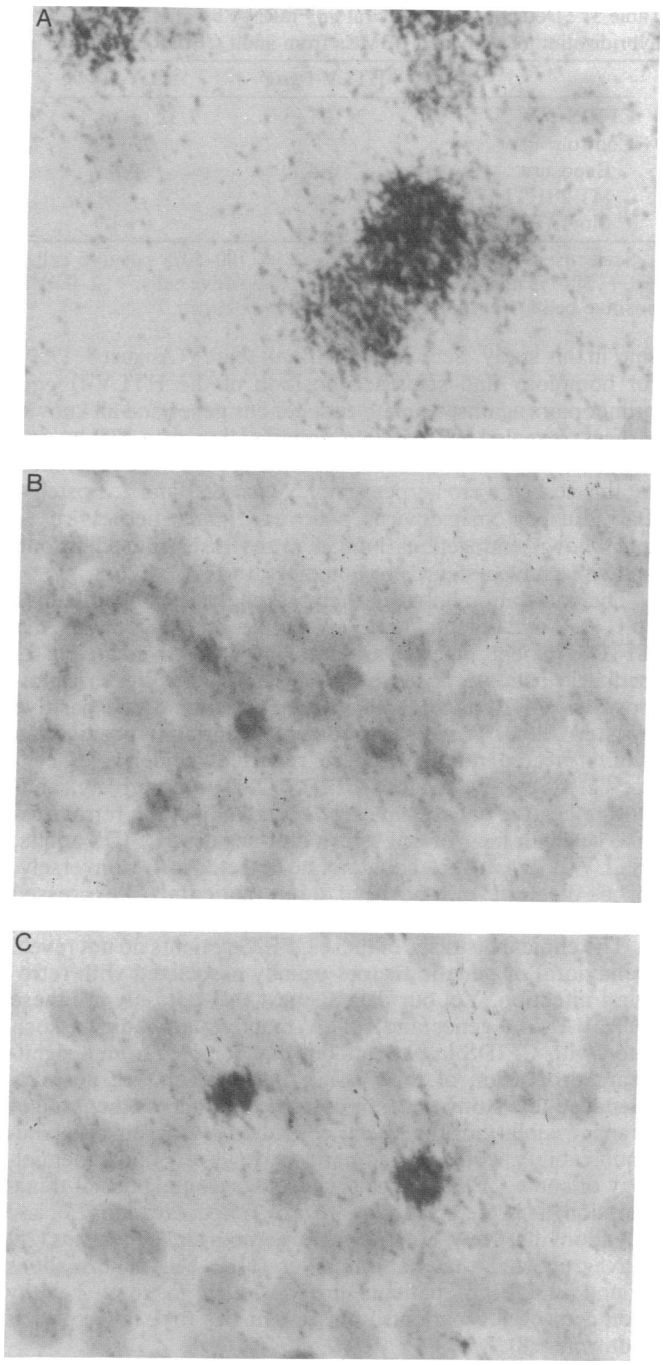

FIG. 5. Detection of HTLV-II *gag* mRNA in activated CFIDS blood cells by *in situ* hybridization. (*A*) Typical viral mRNA-positive pattern of HTLV-II cell reactivity to HTLV-II *gag* is seen in Mo-T cells. (*B*) PBMCs from an exposure control show no virus-positive cells. (*C*) Cells from another CFIDS patient reacted with the HTLV-II *gag* probe.

The HTLV-II-like region we have amplified in CFIDS DNA probably is not a homologous endogenous gene or ubiquitous virus for several reasons. First, endogenous genes are present in all nucleated cells of a positive individual and can be detected by Southern blot hybridization of genomic DNA without the need for prior amplification, as was neces-

Table 3. Detection of retroviral *gag* mRNA by *in situ* hybridization of activated PBMCs from adult CFIDS patients

	HTLV-I *gag*	HTLV-II *gag*
CFIDS	3/12 (+)	7/12 (+ to ++)
Controls		
Exposure	0/4	0/4
MT-2 (HTLV-I)	++++	++
Mo-T (HTLV-II)	−	++++

Scale used to score samples: ++++, 100–50% positive cells; +++, 50–1% positive cells; ++, 1–0.1% positive cells; +, 1–0.01% positive cells; −, <0.01% positive cells.

sary in this study. Second, a GenBank search (August 8, 1990) for homology and sequence position of the HTLV-II *gag* primer pairs against primate endogenous genes and all known viruses revealed no possible amplification of a 409-bp sequence except for HTLV-II *gag*. Among the viruses excluded by this search were herpes simplex viruses 1 and 2, Epstein–Barr virus, cytomegalovirus, poliovirus, human herpesvirus 6, and known endogenous human retroviruses, including one shown to have a degree of homology to HTLV (26).

The correlation between the presence of serum antibodies to HTLV and detectable HTLV-II-like *gag* DNA or RNA in CFIDS patient blood was high but not universal. Of 13 pediatric patients positive by PCR, 10 were Western blot-positive. Of 10 adult PCR-positive patients, 5 had positive Western blots. The lower frequency of antibody-positivity in adults is compatible with the reported incidence of cell-mediated anergy in adult CFIDS (27). Alternatively, antibody-negative patients may reflect latency of the retrovirus-like gene. In fact, of 6 Western blot-negative CFIDS adults, HTLV-II *gag* mRNA could not be detected in 4. Conversely, of 6 Western blot-positive adult patients, 5 expressed HTLV-II *gag* mRNA.

The clinical histories of these CFIDS patients do not reveal behavioral or genetic factors usually associated with retroviral infection. Yet our data suggest that not only are these HTLV-II-like genes and HTLV-reactive antibodies associated with CFIDS in patients but that samples from a significant proportion of their nonsexual contacts are positive. Because this study was not designed to address the issue of transmissibility of the agent, conclusions regarding its communicability would be premature. However, the epidemiology of some CFIDS cases suggests horizontal, casual transmission, as in the epidemics at Lake Tahoe, Nevada (23), and Lyndonville, New York (15). Herberman and colleagues (28) have reported that natural killer (NK) cell abnormalities found in CFIDS patients are observed in some healthy contacts of the patients, but not in healthy, nonexposure controls (28).

Although our data support an association between an HTLV-like agent and CFIDS, we cannot, as yet, define the agent's role in the disease process. Rather than an etiologic agent, it may be a benign secondary infection to which immunologically compromised patients are susceptible. Alternatively, it may be one of two viruses that, when coinfecting the same hematopoetic cells, induce immune dysfunction. In any case, biological characterization of this agent and its role in the pathogenesis of CFIDS awaits its isolation.

We thank Dr. Robert Gallo (National Institutes of Health) for the HTLV-I- and -II-positive T-cell lines MT-2 and Mo-T; Dr. V. Kalyanaraman (Advanced Biosciences, Kensington, MD) for his gift of purified HTLV-I, Dr. Premkumar Reddy (Wistar) for the HTLV-I RNA probe, and Dr. Abbas Vafai (University of Colorado, Denver) for the HTLV-II RNA probe. We are indebted to Drs. Lawrence Seidman and Morton Valow (Philadelphia) for the umbilical cord blood. Advice and helpful discussion was provided by Drs. Rick Mettus and Alagarsamy Srinivasan (Wistar). The technical assistance of Ms. Susan Dorman is appreciated. We thank Mrs. Eva Davis for preparation of the manuscript. This work was supported by National Institutes of Health Grant NS11036 and grants from the Pioneer Foundation and the CFIDS Association.

1. Holmes, G., Kaplan, J., Gantz, N., Komaroff, A., Schonberger, L., Straus, S., Jones, J., Dubois, R., Cunningham-Rundles, C., Pahwa, S., Tosato, G., Zegans, L., Purtilo, D., Brown, N., Schooley, R. & Brus, I. (1988) *Ann. Intern. Med.* **108**, 387–389.
2. Lloyd, A. R., Wakefield, D., Boughton, C. R. & Dwyer, J. M. (1988) *Lancet* **i**, 1286–1287.
3. Swartz, M. N. (1988) *N. Engl. J. Med.* **319**, 1726–1728.
4. Schooley, R. T. (1988) in *Current Clinical Topics in Infectious Diseases*, eds. Remington, J. & Swartz, M. N. (McGraw-Hill, New York), pp. 126–146.
5. Straus, S. E., Dale, J. K., Tobi, M., Lawley, T., Preble, O., Blaese, R., Hallahan, C. & Henle, W. (1988) *N. Engl. J. Med.* **319**, 1692–1698.
6. Acheson, E. D. (1959) *Am. J. Med.* **26**, 569–595.
7. Caligiuri, M., Murray, C., Buchwald, D., Levine, H., Cheney, P., Petersen, D., Komaroff, A. & Ritz, J. (1987) *J. Immunol.* **139**(10), 3306–3313.
8. Kibler, R., Lucas, D., Hicks, M., Poulos, B. & Jones, J. (1985) *J. Clin. Immunol.* **5**, 46–54.
9. Tosato, G., Straus, S., Henle, W., Pike, S. & Blaese, R. (1985) *J. Immunol.* **134**, 3082–3088.
10. Klimas, N. G., Salvato, F. R., Morgan, R. & Fletcher, M. A. (1990) *J. Clin. Immunol.* **28**, 1403–1410.
11. Krueger, G. R. (1987) *Lancet* **19**, 694.
12. Ablashi, D. V., Josephs, S. F., Buchbinder, A., Hellmann, K., Nakamura, S., Llana, T., Lusso, P., Kaplan, M., Dahlberg, J., Memon, S., Imam, F., Ablashi, K., Markham, P., Kramarsky, B., Krueger, G., Biberfeld, P., Wong-Staal, F., Salahuddin, S. & Gallo, R. C. (1988) *J. Virol. Methods* **21**, 29–48.
13. Jones, J., Ray, C., Minnick, L., Hicks, M., Kibler, R. & Lucas, D. (1985) *Ann. Intern. Med.* **102**, 1–7.
14. Yousef, G. E., Bell, E. J., Mann, G. F., Murugesan, V., Smith, D., McCartney, R. & Mowbray, J. (1988) *Lancet* **i**, 146–150.
15. Bell, D. S. & Bell, K. M. (1988) *Ann. Intern. Med.* **109**, 167–173.
16. DeFreitas, E., Wroblewska, Z., Maul, G., Woodward, J., Sheremata, W., Pleasure, D., Grossman, R. & Koprowski, H. (1989) in *HTLV-I and the Nervous System*, ed. Roman, G. (Liss, New York), pp. 407–420.
17. Sambrook, J., Fritsch, E. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
18. Saiki, R. K., Gelfand, D., Stoffel, S., Scharb, S., Higuchi, R., Horn, G., Mullis, K. & Erlich, H. (1988) *Science* **239**, 487–491.
19. McCune, J. M., Namikawa, R., Shih, C., Rabin, H. & Kaneshima, H. (1990) *Science* **247**, 564–568.
20. Harper, M. E., Marcelle, L., Gallo, R. C. & Wong-Staal, F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 772–776.
21. Agius, G., Biggar, R., Alexander, S., Waters, D., Drummond, J., Murphy, E., Weiss, S., Levine, P. & Blattner, W. (1988) *J. Inf. Dis.* **158**, 1235–1244.
22. Williams, A., Fang, C., Slamon, D., Poiesz, B., Sandler, S. G., Darr, W., Shulman, G., McGowan, E., Douglas, D., Bowman, R., Peetoom, F., Kleinman, S., Lenes, B. & Dodd, R. (1988) *Science* **240**, 643–646.
23. Holmes, G., Kaplan, J., Stewart, J., Hunt, B., Pinsky, P. & Schonberger, I. (1987) *J. Am. Med. Assoc.* **257**, 2297–2302.
24. Kalyanaraman, V., Sarngadharan, M., Robert-Guroff, M., Miyoshi, I., Blayney, D., Golde, D. & Gallo, R. (1982) *Science* **218**, 571–573.
25. Hall, W., Kaplan, M., Salahuddin, S., Oyaizu, N., Gurgo, C., Coronesi, M., Nagashima, K. & Gallo, R. in *Human Retrovirology: HTLV*, ed. Blattner, W. A. (Raven, New York), pp. 115–127.
26. Perl, A., Rosenblatt, J., Chen, I., DiVincenzo, J., Bever, R., Poiesz, B. & Abraham, G. (1989) *Nucleic Acids Res.* **17**, 6841–6854.
27. Lloyd, A., Wakefield, D., Broughton, C. & Dwyer, J. (1989) *Med. J. Australia* **151**, 122–124.
28. Ely, N., Grufferman, S., Huang, M., Whiteside, T., Sumaya, C., Saxinger, C. & Herberman, R. (1989) in *Natural Killer Cells and Host Defense*, eds. Ades, E. W. & Lopez, C. (Karger, Basel), pp. 141–145.
29. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3618–3622.
30. Shimotohno, K., Takahashi, Y., Shimizu, N., Gojobori, T., Golde, D., Chen, I. S., Miwa, M. & Sugimara, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3101–3105.