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16. Monoclonal antibodies to EBV and HCMV, and ascites fluid from normal mouse were gifts from G. Pearson, School of Medicine, Georgetown University, Washington, DC. Monoclonal antibodies to VZV and HVS were obtained from N. Chang, Baylor College of Medicine, Houston, TX, and J. Dahlberg, National Cancer Institute, Bethesda, MD, respectively (*15*). HSV-1 and -2 monoclonal antibodies were purchased from Dupont, Boston, MA. Hyperimmune serum to purified African Green and rhesus CMV were prepared in rabbits by D. V. Ablashi. Hyperimmune serum to HVS was a gift from M. Daniel, New England Primate Center, Harvard University, Boston, MA. Some sera from Old World primates were gifts of P. Kanki, Harvard School of Public Health, Boston, MA.
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19. The authors acknowledge C. Cain and T. Barkem for technical assistance, W. Frederick (Howard University School of Medicine, Washington, DC) for clinical specimens, and R. Steinberg of NCI for assistance in preparation of photomicrographs. P.B. was supported by the Swedish Cancer Society of Stockholm, Sweden.

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Genomic Analysis of the Human B-Lymphotropic Virus (HBLV)

STEVEN F. JOSEPHS, S. ZAKI SALAHUDDIN, DHARAM V. ABLASHI, FRANCOIS SCHACHTER, FLOSSIE WONG-STAAL, ROBERT C. GALLO

The human B-lymphotropic virus (HBLV) has a double-stranded DNA genome of greater than 110 kilobase pairs, which is consistent with its morphological classification as a herpesvirus. A 9000–base pair cloned probe of HBLV detected specific sequences in DNA and RNA of infected cells but did not hybridize to the genomic DNA of other human herpesviruses including the Epstein-Barr virus, human cytomegalovirus, herpes simplex type I, and varicella-zoster virus. Conversely, while probes obtained from each of the known human herpesvirus readily detected the homologous viral DNA, they did not hybridize to genomic HBLV DNA. This evidence, in addition to serological and morphological distinctions and the biological effects of this virus demonstrate that HBLV is a novel human herpesvirus.

THE HUMAN B-LYMPHOTROPIC VIrus (HBLV) is a herpes-like virus on the basis of its icosahedral core structure of 162 capsomers. However, it has several unusual features in terms of host-range, morphology, and serology (*1*) that distinguish it from other known members of the human herpesvirus family [the Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), herpes simplex type 1 and type 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV)] (*2*). We report here that HBLV contains a large molecular weight, double-stranded DNA genome that is distinct from the DNA of known human herpesviruses by Southern blot analyses.

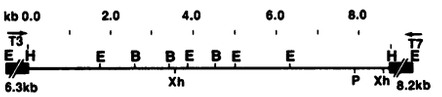

Fig. 1. A restriction map of the HBLV clone pZVH14 obtained as described (*3*). The insert size is 9.0 kb. Shown are the restriction enzyme sites for Bam HI (B), Eco RI (E), Xho I (Xh), Pst I (P), and Hind III (H). The dark boxes indicate the position and size of flanking viral Eco RI fragments detected in genomic viral and infected cellular DNA. The positions of the T3 and T7 promoters contained in the vector are shown by the labeled arrows.

S. F. Josephs, S. Z. Salahuddin, F. Schachter, F. Wong-Staal, R. C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20894.
D. V. Ablashi, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20894.

The isolation of HBLV was performed by cocultivation of peripheral blood lymphocytes (from patients) with phytohemagglutinin-stimulated human umbilical-cord blood lymphocytes and by cell-free transmission of virus from HBLV-positive patients. Propagation was continued by reinfection of fresh umbilical-cord blood cells with filtered (0.45-μm filters) culture supernatants as described (*1*). The supernatants were then layered onto 20% glycerol cushions and centrifuged at 100,000*g* for 3 hours and the DNA ($HBLV_{dp}$ DNA) was extracted from the pelleted virions (*3*).

A molecular clone designated pZVH14 (Fig. 1) was then obtained from the $HBLV_{dp}$ DNA (*3*). When used as a probe, this clone specifically hybridized to DNA of HBLV-infected human umbilical-cord blood cells harvested after several rounds of cell-free virus transmission (Fig. 2) and to the cytoplasmic messenger RNA (mRNA) of infected cells but not to DNA or RNA of uninfected cells (*1*).

The pZVH14 probe was also useful for detection of virus in sucrose gradients by quantitative DNA dot-blot analyses (*4*). Two major peaks were evident (Fig. 3). The first was in fractions 3–5 (density 1.13 g/ml), which was probably enveloped virus; and the second was the pellet, which probably contained the unenveloped capsids. Fractions 3–5, which contained the majority of the virus, were pooled and used to obtain DNA from gradient-banded virions ($HBLV_{bv}$ DNA) (*5*). Bam-HI digests of end-labeled (*6*) $HBLV_{bv}$ DNA indicate a minimum molecular weight estimate for HBLV of >110,000 base pairs (data not shown).

To determine whether there is any significant homology of HBLV to other human herpesviruses we performed Southern blot hybridizations (*7*) with two sets of filters (Fig. 4, A and B) that contained the restriction enzyme–treated genomic DNA's (*8, 9*) of $HBLV_{bv}$, $HBLV_{dp}$, CMV, HSV-1, EBV, VZV, and *Herpesvirus saimiri* (HVS) of squirrel monkeys. Each set of filters was hybridized to a ^{32}P-labeled viral DNA probe (*8, 9*), then washed and analyzed by autoradiography. The probe was then removed by boiling the filters before each successive hybridization. In some of the rehybridized filters, weak bands could be seen as a result of incomplete removal of the previously hybridized probe. These proved useful in aligning the filters and as convincing reference points for the new bands that appeared after each new hybridization. Set A was first hybridized to the HBLV probe pZVH14

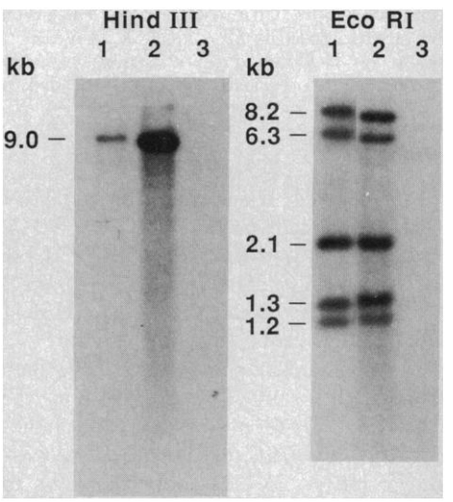

Fig. 2. Hybridization of HBLV clone pZVH14 to DNA's digested with Hind III and Eco RI (*5*) (Fig. 5). Clone pZVH14 hybridized specifically to extracellular virus DNA ($HBLV_{dp}$ DNA) (lanes 1) or infected umbilical-cord blood cell DNA (lanes 2), but not to the uninfected control DNA isolated from the skin of an AIDS patient (lanes 3). The infected cell DNA shown in lane 2 was isolated after several rounds of cell-free virus transmission in human umbilical-cord blood cells.

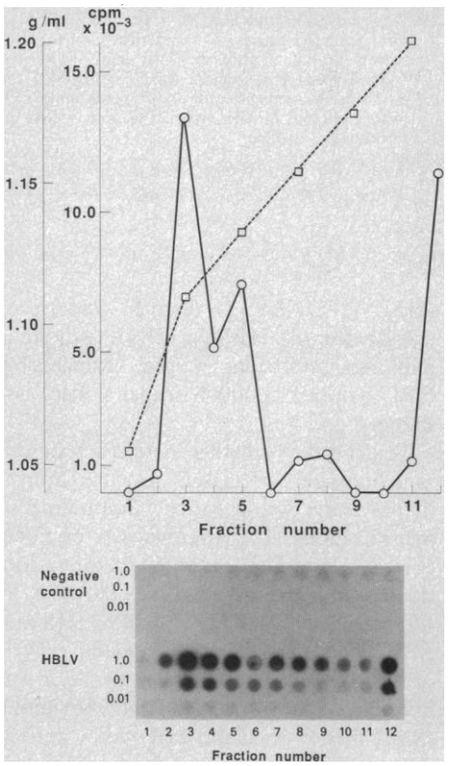

Fig. 3. DNA dot-blot analysis of sucrose-banded HBLV. Frozen supernatants (80 ml) from virus-infected umbilical-cord lymphocyte cultures were pooled and clarified by centrifugation at 8000*g* for 2 minutes. The virus was pelleted from the clarified supernatants at 17,000 rev/min in a Beckman SW28 rotor for 90 minutes. The pellets were resuspended in TNE and layered onto a 15–60% sucrose gradient that was centrifuged for 30 minutes at 20,000 rev/min in a Beckman SW41 rotor. Fractions were collected from the top in 1-ml volumes. These were diluted to 10 ml with TNE and centrifuged at 17,000 rev/min for 90 minutes in a Beckman SW41 rotor. The pellets were resuspended in 50 μl of TNE, and 5 μl of each was used for dot-blot analysis (*4*) with pZVH14. Incorporated ^{35}S [○–○] and density [□–□] of the fractions were measured.

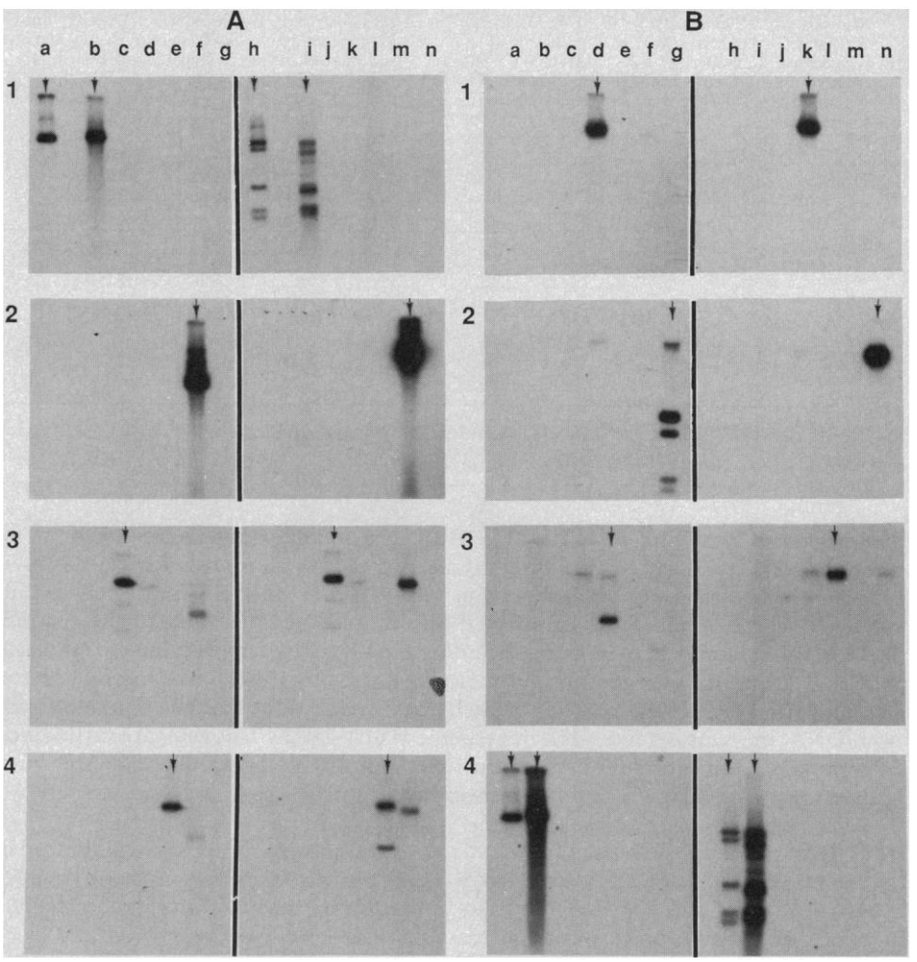

Fig. 4. Southern blot hybridizations (*7*) to compare HBLV DNA to DNA from known human herpesviruses (*8, 9*). Blotted filters contained $HBLV_{bv}$ (lanes a, h), $HBLV_{dp}$ (lanes b, i), HCMV (lanes c, j), HSV-1 (lanes d, k), EBV (lanes e, l), VZV (lanes f, m), and HVS (lanes g, n). Lanes a–g contain DNA's digested with Hind III and lanes i–m contain the Eco RI samples. Two sets of filters (A and B) were each hybridized to a probe, washed, exposed, and then boiled for 5 minutes in water before rehybridizing with the next probe. The ^{32}P-labeled probes for set A were pZVH14 (A1), VZV Bam–E (A2), CMV Eco RI–A pHD-1 (A3) and EBV Bam HI–C (A4). The ^{32}P-labeled probes for set B were HSV-1 pSG18FB50 (B1), HVS pT7.4 (B2), EBV Bam HI–K (B3), and HBLV pZVH14 (B4). Hybridizations were carried out with purified probes except for the HCMV Eco RI–A pHD-1 probe and the EBV Bam HI–K probe, both of which contained plasmid vector sequences. Each filter was hybridized for 15 hours at 37°C in 2 ml of hybrid solution containing 3× SSC, 50% formamide, 0.02% sodium dodecyl sulfate (SDS), 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, yeast transfer RNA (62.5 μg/ml), 10% dextran sulfate, and 8×10^6 cpm of probe (specific activity after nick-translation, 10^8 cpm/μg). After hybridization, the filters were washed three times at room temperature in 1× SSC, 0.5% SDS, then incubated for 30 minutes at 60°C, and rinsed three times with washing buffer at room temperature, blotted to dampness, and exposed at room temperature for 1 hour (except for A2 which was exposed 10 minutes) in cassettes equipped with Quanta III (Dupont) screens. Arrows indicate lanes showing the hybridization where DNA on the filter and the probe DNA represented the same virus.

(A1) followed in succession by the VZV Bam E fragment probe (A2), the HCMV Eco RI A fragment probe (A3), and the EBV Bam HI C fragment probe (A4). Set B was first hybridized to the HSV-1 pSG18FB50 probe (B1), then to an HVS probe pT7.4 (B2), thirdly to an EBV Bam K fragment probe (B3), and finally to the HBLV pZVH14 probe (B4). Autoradiograms A1 and B4 showed the bands predicted for HBLV ZVH14 probe according to the map (Fig. 1) for the homologous DNA's. They are 9.0 kb for Hind III and (from top to bottom), 8.2, 6.3, 2.1, 1.3, and 1.2 kb for Eco RI. No cross-hybridizations of the HBLV probe ZVH14 occurred to any of the other herpesviruses examined. Conversely, while they hybridized to the homologous genomic DNA, none of the other viral probes hybridized to the lanes containing $HBLV_{dp}$ DNA or $HBLV_{bv}$ DNA digested with either Eco RI or Bam HI. Even though the HSV-2 genome was not present on the blots, cross-hybridization would have occurred between the HSV-1 probe pSG18F-B50 and HSV-2 under the moderately stringent washing conditions used [1× SSC (standard saline citrate) 0.5% SDS, 60°C, 30 minutes] (*10*). Indeed, some cross-hybridization was seen between the HSV-1 probe pSG18F-B50 and VZV (Fig. 4, B1, lanes f and m).

We conclude that HBLV is distinct from other previously characterized human herpesviruses (and HVS) by nucleic acid analyses presented here in addition to the previous serological, morphological, and biological (*1*) results.

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3. For the isolation of $HBLV_{dp}$ DNA, supernatants from HBLV-infected umbilical-cord blood cells were layered onto 20% glycerol cushions and centrifuged at 30,000 rev/min for 3 hours in a Beckman SW41 rotor at 4°C. The pellets were suspended in TNE buffer (10 m*M* tris-HCl, *p*H 9; 100 m*M* NaCl; 1 m*M* EDTA), and extracted with PCI9 (phenol:chloroform:isoamyl alcohol:tris-HCl, *p*H 9::100:100:1 by volume) followed by extraction with chloroform–isoamyl alcohol (24:1 by volume). Nucleic acids were precipitated by addition of two volumes of 95% ethanol ($HBLV_{dp}$ DNA). $HBLV_{dp}$ DNA was digested with Hind III and cloned into the Bluescribe vector (Vector Cloning Systems). Several clones obtained after screening with nick-translated (^{32}P-labeled) $HBLV_{dp}$ DNA as a probe were examined for specificity of hybridization to infected human umbilical-cord blood cell DNA and by in situ hybridization to cytoplasmic RNA.
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5. For the isolation of $HBLV_{bv}$ DNA, the gradient-banded virions were lysed by addition of SDS to 1% and proteinase K to 100 μg/ml and incubated at 37°C for 30 minutes and then at 65°C for 5 minutes. One volume of PCI9 was added and the mixture gently shaken for 5 minutes and then centrifuged in an Eppendorf centrifuge at 4°C for 15 minutes. The supernatant was removed, and extracted with one volume of chloroform–isoamyl alcohol (24:1) and centrifuged for 5 minutes. The aqueous layer was removed and the DNA precipitated by addition of 2 volumes of ethanol. The DNA was dissolved in 50 μl of water. The DNA recovered amounted to approximately 1 μg.
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8. AD-169 strain of human CMV was used to infect secondary cultures of fibroblastic cells (Flow 7000) when the cells were 85% confluent. The cells were harvested to extract DNA when approximately 25% of cells showed cytopathic effects. The infected cells were also tested by indirect immunofluorescence assays (IFA) with CMV antibody–positive human serum and monoclonal antibodies (*1*). Similarly, Flow 7000 cells were also infected with HSV-1 (J.W. strain, kindly supplied by Dr. B. Hampan). The infected cells were harvested as described for CMV. The infected cells were positive in the IFA, as measured with human serum and monoclonal antibodies. Human cord blood mononuclear cells were used for HBLV infection (*1*). The supernatants from the infected cells were harvested when greater than 80% of the cells were IFA-positive with HBLV antiserum. Purification of HCMV and HSV-1 was on 15–30% sucrose gradients according to the method of Bornkamm *et al.* [G. W. Bornkamm, H. Delius, B. Fleckenstein, F.-J. Werner, C. Mulder, *J. Virol.* **19**, 154 (1976)] prior to extracting the DNA.
9. The HSV-1 probe pSG18F-B50 was a gift of R. Robinson of the University of Texas Health Science Center, Dallas, TX. The VZV Bam HI–E fragment probe and VZV genomic DNA were gifts of S. Strauss of NIAID, NIH, Bethesda, MD. The HVS probe pT7.4 and HVS genomic DNA were gifts of R. Desrosier [R. C. Desrosier, R. L. Burghoff, A. Bakker, J. Kamine, *ibid.* **49**, 343 (1984)]. The CMV Eco RI–A fragment (pHD-1) probe was a gift of E. S. Huang [M. G. Davis, E. C. Mar, Y.-M. Wu, E.-S. Huang, *ibid.* **52**, 129 (1984)]. The EBV Bam HI–K fragment (strain B 95-8) was a gift of M. Nonayamo [G. Miller, T. Shope, H. Lisco, P. Stitt, M. Kipman, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 383 (1972)]. The EBV Bam HI–C fragment was provided by N. T. Chang of Baylor School of Medicine, Houston, TX. EBV genomic DNA was purchased from Showa University Research Institute for Biomedicine in Florida, St. Petersburg, FL.
10. R. Robinson, personal communication.
11. We thank N. T. Chang (Baylor School of Medicine) and E. Kieff (University of Chicago) for helpful discussion, and Dr. L. G. Chatlynne (PDS Laboratories) for assistance in the experiments.

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Trypanosoma cruzi Infection Inhibited by Peptides Modeled from a Fibronectin Cell Attachment Domain

M. A. Ouaissi, J. Cornette, D. Afchain, A. Capron, H. Gras-Masse, A. Tartar

The mechanism by which *Trypanosoma cruzi*, the protozoan parasite that causes Chagas' disease, becomes attached to mammalian cells is not well understood. Fibronectin is thought to participate in the attachment, and in this study the region of fibronectin that interacts with the surface receptors of *T. cruzi* trypomastigotes was investigated by testing the binding of the amino acid sequence Arg-Gly-Asp-Ser, corresponding to the cell attachment site of fibronectin to *T. cruzi* trypomastigotes. Peptides with the sequence Arg-Gly-Asp-Ser, but not Arg-Phe-Asp-Ser, Arg-Phe-Asp-Ser-Ala-Ala-Arg-Phe-Asp, Ser-Lys-Pro, Glu-Ser-Gly, or Ala-Lys-Thr-Lys-Pro, bound to the parasite surface and inhibited cell invasion by the pathogen. Monoclonal antibodies to the cell attachment domain of fibronectin also inhibited cell infection by the parasite. The immunization of BALB/c mice with tetanus toxoid–conjugated peptide induced a significant protection against *T. cruzi*. The data support the notion that the sequence Arg-Gly-Asp-Ser of cell surface fibronectin acts as a recognition site for attachment of the parasites.

Attachment of *Trypanosoma cruzi*, the causative agent of Chagas' disease, to mammalian cells involves the interaction of the plasma membranes of the pathogen and the host cell. This interaction is believed to be of fundamental importance in the host cell invasion by the pathogen. Investigators searching for molecules that might mediate parasite-cell interactions believe that the receptor or receptors on host cells for parasite binding are glycoproteins (*1*). However, little is known about their nature.

Fibronectin (Fn), which is a high molecular weight glycoprotein composed of 220-kD subunits linked into dimers and polymers by disulfide bonds, is present in blood and connective tissue and at cell surfaces (*2*). It participates in a number of cell surface interactions with the local extracellular microenvironment (*3, 4*). Fibronectin can mediate the attachment of pathogens such as *Treponema pallidum* (*5*) and *Leishmania* species (*6*) to the host cell surface. Recently, we demonstrated that Fn interacts with *T. cruzi* trypomastigote surface receptor (*7*). In addition, involvement of this Fn receptor in the interaction between *T. cruzi* and vertebrate cells has been reported (*8*).

The Fn molecule consists of highly structured domains containing the binding sites for the macromolecules that interact with it (*9*). The site of its attachment to mammalian cells has been characterized (*10*), and the analysis of small synthetic peptides has shown that this recognition site is carried by the sequence Arg-Gly-Asp-Ser (*11*). The region of Fn that interacts with the surface of the trypomastigote, the infective stage of the parasite, is unknown. We used monoclonal antibodies to the cell-attachment site in Fn (*10*), as well as synthetic peptides modeled from the sequence of this domain (Table 1), to show that this dimeric protein acts as a bridge between the cell surfaces of host cell and *T. cruzi* with the same hydrophilic sequence Arg-Gly-Asp-Ser of Fn participating in these interactions.

Analysis of the data in Fig. 1 indicates that the infectivity of 3T3 fibroblasts by *T. cruzi* can be inhibited by monoclonal antibodies to Fn. The antibodies M1205 and 3E3 inhibited cell invasion by the parasite at antibody concentrations greater than 0.1 mg/ml, whereas 4B2 was inhibitory only at high concentrations.

Both 3E3 and 4B2 reacted with the 120-kD chymotryptic fragment of Fn. However, fractionation of a pepsin digest of this fragment allowed the identification of a large fragment of 50 kD that binds to a 4B2-adsorbed Sepharose column and a small one (15 kD) that binds to 3E3-adsorbed Sepharose. Biological activity that promoted cell attachment was found in the 15-kD peptide (*10*). On account of the inhibitory effect of the monoclonal antibody 3E3 on cell inva-

M. A. Ouaissi, J. Cornette, D. Afchain, A. Capron, Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM U. 167-CNRS 624, 59019 Lille Cedex, France.
H. Gras-Masse and A. Tartar, Service de Chimie des Biomolécules, JE CNRS, Institut Pasteur de Lille, 59019 Lille Cedex, France.