

Viral Coinfections among African Children Infected with Human Immunodeficiency Virus Type 1

Rana Chakraborty,^{1,4} Gareth Rees,² Dimitra Bourboulia,³ Alexandra M. Cross,¹ Jedediah R. Dixon,¹ Angelo D'Agostino,⁴ Rachel Musoke,⁴ Chris Boshoff,³ Sarah L. Rowland-Jones,¹ and Paul Klenerman²

¹Human Immunology Unit, Weatherall Institute of Molecular Medicine, and ²Peter Medawar Building for Pathogen Research, University of Oxford, Oxford and ³Wolfson Institute for Biomedical Research, University College London, London, United Kingdom; and ⁴Nyumbani Children's Home, Nairobi, Kenya

City-dwelling children from Kenya who were infected with human immunodeficiency virus type 1 (HIV-1) were tested for coinfection with cytomegalovirus (CMV), human T cell lymphotropic viruses 1 and 2, Kaposi sarcoma-associated herpesvirus (KSHV), or hepatitis B, C, and G viruses. All children were found to be coinfecting with CMV, whereas 5% had hepatitis G virus coinfection and 15% had KSHV coinfection. A protective role for hepatitis G virus cannot be excluded but likely affects only a minority of HIV-1-infected African children.

Despite substantial progress in the development of strategies for the prevention of transmission of HIV-1 from mother to child, the transmission of HIV-1 infection to infants has continued to increase globally, particularly in resource-poor settings. The rate of vertical transmission of HIV-1 in African countries is 25%–42%. An estimated 600,000 new pediatric infections occur each year, of which ~1500 per day (>90%) occur in sub-Saharan Africa [1].

Although only 4% of the world's population of HIV-infected subjects are children, 20% of all deaths due to AIDS have occurred among children. Early studies of children perinatally infected with HIV-1 before the era of HAART have indicated

that ~25% of these children had very rapid progression of HIV-1 infection to AIDS (within the first year of life). For the remaining 75% of perinatally infected children, the median time to progression to AIDS was ~7 years [2]. Biological determinants that contribute to accelerated disease progression may include relative immunologic immaturity, thymic HIV-mediated destruction during active thymopoiesis, and sharing of human leukocyte antigen class 1 between mother and infant [2]. Pre- or postnatal acquisition of viral coinfections may influence disease progression. HIV-1-infected infants who acquire cytomegalovirus (CMV) infection within the first 18 months of life have a significantly higher rate of HIV-1 disease progression and CNS disease than do those who are infected with HIV-1 alone [3]. Furthermore, the hepatitis C virus (HCV) RNA concentration has been independently associated with disease progression [4]. In contrast, HIV-1-infected adults with previous or current infection with hepatitis G virus (HGV) may have higher CD4⁺ T cell counts and better AIDS-free survival rates [5]. The role of Kaposi sarcoma-associated herpesvirus (KSHV) in HIV-1 disease progression is not yet clear, although KSHV-derived proteins may potentially up-regulate HIV replication [6].

There are few studies documenting the seroprevalence rates of viral coinfections among HIV-1-infected children in Africa. We therefore determined the prevalence of coinfection with KSHV, CMV, human T cell lymphotropic viruses 1 and 2 (HTLV-1 and -2), or hepatitis B, C, and G virus in a cross-sectional study of all HIV-1-infected children (age range, 1–18 years) residing at Nyumbani Children's Home in Nairobi, Kenya.

Subjects and methods. In 2000, Nyumbani Children's Home provided care for 71 HIV-1-infected children who were referred by hospital social workers when no primary caregiver for the children could be identified. The children were accepted into the home at different ages, depending on the availability of space. The median age of the children at the time of entry into the home was 2.2 years (range, 0–12.7 years). Medical documentation (including data on the mode of HIV-1 transmission) was often incomplete before the children were admitted to the home on the basis of the absence of a caregiver.

It was assumed that all children were infected in utero, during labor, or by transmission of HIV-1 through breast milk. A diagnosis of HIV-1 infection was established and confirmed by testing of successive serum samples for HIV-1 antibodies by use of commercial ELISA. For children <18 months of age, the number of plasma HIV-1 RNA copies, as determined by

Received 24 September 2002; accepted 4 December 2002; electronically published 19 March 2003.

Financial support: R.C. is a Medical Research Council (UK) Clinical Training Fellow; S.L.R.-J. holds an Elizabeth Glaser Scientist award from the Pediatric AIDS Foundation; and P.K. is a Wellcome Trust Senior Clinical Fellow.

Reprints or correspondence: Dr. Rana Chakraborty, St. George's Hospital and Medical School, Paediatric Infectious Diseases Unit, 5th Floor Lanesborough Wing, Blackshaw Rd., Tooting, London SW17 0QT, United Kingdom (ranachakraborty@hotmail.com).

Clinical Infectious Diseases 2003;36:922–4

© 2003 by the Infectious Diseases Society of America. All rights reserved.
1058-4838/2003/3607-0015\$15.00

RT-PCR, confirmed diagnosis of HIV-1 infection. Routine care included adequate nutrition, prophylactic cotrimoxazole given 3 times weekly, and immunizations administered in line with the recommendations of the World Health Organization Expanded Program.

The children's home, by default, selected for typical and slow progressors. Rapid progressors with early-onset *Pneumocystis carinii* pneumonia would have died in infancy before adoption was possible. This was reflected in the favorable survival outcomes (a 70% survival rate for children residing in the home at age 3 years vs. an 11% survival rate for HIV-1-infected children aged 3 years from community settings in Blantyre, Malawi [7]).

"Long-term survivors" (LTSs) were defined as children who reached the age of 8 years without receiving antiretroviral therapy and who were subclassified as either LTS nonprogressors (LTSNPs; i.e., children with a CD4 T cell count of ≥ 500 cells/mm³) or LTS progressors (LTSPs; i.e., children with a CD4 T cell count of < 500 cells/mm³). Children < 8 years of age were subclassified, on the basis of age-specific CD4 T cell counts, either as early nonprogressors with category 1 infection, according to the Centers for Disease Control and Prevention (CDC; Atlanta, GA) classification of pediatric HIV-1 infection, or as typical progressors with category 2 or 3 infection, according to the same CDC classification.

Informed consent was obtained from the legal guardian of the children. Research ethics committees at the University of Oxford (Oxford, United Kingdom) and the National Council of Science and Technology in Nairobi, Kenya approved all studies.

Asymptomatic children were screened using a commercial ELISA kit (Murex) for the detection of IgG antibodies to HTLV-1 and -2 and to CMV. Antibodies against latent nuclear antigen 1 of KSHV were detected using an indirect IFA, as described elsewhere [8]. Asymptomatic children were also screened for hepatitis B surface antigen (HBsAg) by use of an Abbott AxSYM analyzer. Samples that were positive or borderline positive for HBsAg were rechecked by neutralization assay performed according to the manufacturer's instructions.

HCV detection was performed using nested RT-PCR on extracted RNA (Quiagen viral RNA Minikit). The primer set 5'-ggTgCACggTCTACgAgCCT-3' and 5'-CCCTgTgAggAACT(A/T)CTgTCTTCACGC-3' was used for the first round, and the primer set 5'-CACTCgCAATgCACCTATCaggCgT-3' and 5'-TCTAgCCATggCgTTAgT(A/g)(C/T)gAgTgT-3' was used for the second round. HGV/GB virus-C was analyzed using seminested RT-PCR modified from a method described elsewhere [9]; primers 77F and 211R were used. A third primer (AS2) was included in the second round with the following sequence: 5'-gTCTTCTCATCTgAgCTgCTCT-3'.

Results. All children were found to be seropositive for

CMV IgG antibodies by ELISA. CMV viraemia was detected, by DNA hybridization assay, in 8 (15%) of 55 children, which suggests the presence of primary or reactivated CMV infection [10]. Of these 8 children, 5 were typical progressors, 1 was an early nonprogressor, 1 was an LTSP, and 1 was an LTSNP. None of the subjects was given a clinical diagnosis of HIV encephalopathy, retinitis, pneumonitis, or colitis.

Nine (15%) of 61 patients were seropositive for KSHV (table 1). One KSHV-seropositive sample had been obtained from a child who had died and who was found, at autopsy, to have Kaposi sarcoma (KS) within the mesenteric lymph nodes [11]. No living child had evidence of KS. Titers of antibodies to HTLV-1 and -2 were not detected among any of the children ($n = 59$).

HGV RNA was detected in 3 (5%) of 60 subjects tested (1 LTSNP, 1 LTSP, and 1 typical progressor). HBsAg was reactive in 2 (4%) of 54 children (table 1), and HCV RNA was not detected in any of the 60 patients.

Discussion. There is a paucity of data about the natural history of viral coinfections among HIV-1-infected African children infected vertically or by the perinatal route. The present observational study is the first study to report the seroprevalence rates of some key viral coinfections among a select group of subjects.

Given the cross-sectional design and power of this study, we were unable to draw conclusions as to the influence of viral coinfections on HIV-1 disease progression. Of interest, 2 of 3 children coinfecting with HGV and HIV-1 were LTSs aged > 10 years. However, among African children, the influence of HGV on HIV-1 disease nonprogression in the cohort overall would appear to be small, and other factors must be sought to explain the differences in HIV-1 disease progression rates.

Our observations reflect the findings of Liu et al. [12] in Kinshasa, Democratic Republic of Congo, who documented the higher seroprevalence of HGV/GBV-C infection among pregnant women, compared with the seroprevalences of HCV

Table 1. Coinfection status of HIV-1-infected African children, according to HIV-1 disease progression classification.

Classification	No. of children with a positive result/ no. of children assessed			
	CMV viraemia	HGV RNA	HBsAg	KSHV seropositive
Early nonprogressor	1/5	0/5	1/5	0/5
Typical progressor	5/26	1/28	0/23	1/30
LTSNP	1/17	1/17	0/17	4/16
LTSP	1/7	1/10	1/9	4/10
Total, n/N (%)	8/55 (15)	3/60 (5)	2/54 (4)	9/61 (15)

NOTE. CMV, cytomegalovirus; HGV, hepatitis G virus; HBsAg, hepatitis B surface antigen; KSHV, Kaposi sarcoma-associated herpesvirus; LTSNP, long-term survivor nonprogressor; LTSP, long-term survivor progressor.

and HTLV-1. The authors concluded that HGV/GBV-C was more readily transmitted than was HCV. In addition, de Martino et al. [13], who followed 58 HIV-1-infected mothers and their infants, concluded that mother-to-child transmission of HIV-1 is frequent without evidence of liver disease. Perinatal transmission patterns and clinical observations from our cohort support these conclusions. The observed frequencies of viral coinfection in the children within the cohort that was studied may reflect the transmission biology of these different viruses and their prevalence in the maternal population. The prevalence of HCV in this part of Africa is thought to be fairly low, although few data on this subject exist [14].

Coinfection with KSHV was not infrequently detected among the children. Disseminated KS was identified within the mesenteric lymph nodes of one child who had high titers of KSHV IgG. Seroprevalence data indicate considerable differences in rates of infection with KSHV when Western populations are compared with populations from East Africa [15]. This may account for the significant disparity in the prevalence of this malignancy among immunocompromised children. Increased seroprevalence of KSHV in older subjects (8 of 9 KSHV-seropositive children were >8 years of age) may reflect a cohort effect from progressive nonsexual horizontal transmission among a group of children residing communally within an area of endemicity [16].

The present report documents the prevalence of viral coinfections associated with morbidity and mortality among HIV-1-infected children. An earlier study from our group concluded that pyogenic infections were the most common cause of death among children residing in the Nyumbani Children's home, although disseminated CMV infection and KS were reported as incidental findings in 2 children [11]. More research is needed in resource-poor settings with larger cohorts, which also account for the influence of other microbial infections. The body of evidence that is currently emerging may further elucidate the dynamics of HIV-1 infection among poorly characterized and vulnerable populations.

Acknowledgments

We thank Jennifer Tossbill at the Public Health and Laboratory Services in London as well as Simon Ogola and all the staff and children at Nyumbani Children's Home.

References

1. Joint United Nations Programme on HIV/AIDS. AIDS epidemic update-December 2001: new HIV estimates and additional data. Available at: http://www.unaids.org/epidemic_update/report_dec01/index.html. Accessed April 2002.
2. Goulder PJ, Jeena P, Tudor-Williams G, Burchett S. Paediatric HIV infection: correlates of protective immunity and global perspectives in prevention and management. *Br Med Bull* **2001**;58:89-108.
3. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. *N Engl J Med* **1999**;341:77-84.
4. Daar ES, Lynn H, Donfield S, et al. Hepatitis C virus load is associated with HIV-1 disease progression in hemophiliacs. *J Infect Dis* **2001**;183:589-95.
5. Yeo AE, Matsumoto A, Hisada M, Shih JW, Alter JW, Goedert JJ. Effect of hepatitis G virus infection on progression of HIV infection in patients with hemophilia. Multicenter Hemophilia Cohort Study. *Ann Intern Med* **2000**;132:959-63.
6. Hyun TS, Subramanian C, Cotter MA 2d, Thomas RA, Robertson ES. Latency-associated nuclear antigen encoded by Kaposi's sarcoma-associated herpesvirus interacts with Tat and activates the long terminal repeat of human immunodeficiency virus type 1 in human cells. *J Virol* **2001**;75:8761-71.
7. Taha TE, Graham SM, Kumwenda NI, et al. Morbidity among HIV-1-infected and -uninfected African children. *Pediatrics* **2000**;106:e77-86.
8. Wilkinson J, Cope A, Gill J, et al. Identification of Kaposi's sarcoma-associated herpesvirus (KSHV)-specific cytotoxic T-lymphocyte epitopes and evaluation of reconstitution of KSHV-specific responses in human immunodeficiency virus type 1-infected patients receiving highly active antiretroviral therapy. *J Virol* **2002**;76:2634-40.
9. Linnen J, Wages J Jr, Zhang-Keck Z-Y, et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* **1996**;271:505-8.
10. Mansy F, Brancart F, Liesnard C, Bollen A, Godfroid E. A PCR based DNA hybridisation capture system for the detection of human cytomegalovirus: a comparative study with other identification methods. *J Virol Methods* **1999**;80:113-22.
11. Chakraborty R, Pulver A, Pulver LS, et al. The post-mortem pathology of HIV-1-infected African children. *Ann Trop Paediatr* **2002**;22:125-31.
12. Liu HF, Muyembe-Tamfum JJ, Dahan K, Desmyter J, Goubau P. High prevalence of GB virus C/hepatitis G virus in Kinshasa, Democratic Republic of Congo: a phylogenetic analysis. *J Med Virol* **2000**;60:159-65.
13. de Martino M, Azzari C, Resti M, et al. Hepatitis G virus infection in human immunodeficiency virus type 1-infected mothers and their children. *J Infect Dis* **1998**;178:862-5.
14. Heintges T, Wands JR. Hepatitis C virus: epidemiology and transmission. *Hepatology* **1997**;26:521-6.
15. Boshoff C, Weiss RA. Epidemiology and pathogenesis of Kaposi's sarcoma-associated herpesvirus. *Philos Trans R Soc Lond B Biol Sci* **2001**;356:517-34.
16. Gessain A, Mauclore P, van Beveren M, et al. Human herpesvirus 8 primary infection occurs during childhood in Cameroon, Central Africa. *Int J Cancer* **1999**;81:189-92.