© Med Sci Monit, 2011; 17(3): CR154-158
PMID: 21358602

WWW.MEDSCIMONIT.COM
Clinical Research

Received: 2009.02.24
Accepted: 2010.10.14
Published: 2011.03.01

Confirmation of HIV-like sequences in respiratory tract bacteria of Cambodian and Kenyan HIV-positive pediatric patients

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Vladimir Zajac[1,2ADEF], Lenka Matelova[1ABC], Anna Liskova[2B], Michal Mego[3ABC], Vladimir Holec[3AEF], Zuzana Adamcikova[1,2ABC], Viola Stevurkova[1AEF], Andrea Shahum[2BG], Vladimir Krcmery[2BG]

[1] Department of Cancer Genetics, Cancer Research Institute, Bratislava, Slovakia
[2] St. Elizabeth University College of Health and Social Sciences, Bratislava, Slovakia (St Maximilian Kolbe Tropical Programme, Phnom Penh, Cambodia)
[3] Comenius University, Medical Faculty, Bratislava, Slovakia

Source of support: This work was supported by the Grant No. APVV-0404-07 from the APVV Grant Agency and by the Grant No. 2/5025/25 from VEGA Grant Agency of Slovak Republic

Summary

Background: Bacteria and yeasts isolated from respiratory tracts of 39 Cambodian and 28 Kenyan HIV-positive children were tested for the presence of HIV-1 sequences.

Material/Methods: Bacteria and yeasts from the respiratory tract (nose, pharyngeal swabs) were isolated from 39 Cambodian and 28 Kenyan HIV-positive children. Bacterial chromosomal DNA was prepared by standard protocol and by Qiagen kit. The PCR specific for HIV sequences was carried out using HIV-1-specific primers.The analysis was performed by colony and dot-blot hybridization using HIV-1-specific primers which represent *gag*, *pol* and *env* genes of the virus. The sequencing of some PCR products was performed on the ABI 373 DNA Sequencer.

Results: The majority of microbes were characterized as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and resp. *Candida albicans*. In some cases *E. coli*, *Streptococcus pyogenes*, *Proteus mirabilis* and *Candida tropicalis* were identified. Bacteria of 16 Cambodian (41%) and 8 Kenyan (31%) children were found to be positive in colony and dot-blot DNA hybridization. By the sequencing of PCR products synthesized on the template of patients' bacterial DNA using primers 68;69 for env HIV-1 gene, homology of greater than 90% with HIV-1 isolate HXB2 (HIVHXB2CG) was revealed.

Conclusions: Bacteria and yeasts from the respiratory tract of 41% of Cambodian and 31% of Kenyan HIV-positive children bear HIV-like sequences. The role of bacteria in the HIV disease process is discussed.

key words: **respiratory tract bacteria • HIV/AIDS positive children • HIV-like sequences • DNA hybridization • GALT, viral reservoir**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=881449

Word count: 1669
Tables: 2
Figures: 3
References: 18

Author's address: Vladimír Zajac, Department of Cancer Genetics, Cancer Research Institute, Slovak Academy of Sciences, Vlarska 7 Str., 83391 Bratislava, Slovak Republic, e-mail: vladimir.zajac@savba.sk

Background

It has recently been found that both acute human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infections are accompanied by a dramatic and selective loss of memory $CD4^+$ T cells, predominantly from the mucosal surfaces [1].

Gut-associated lymphatic tissue (GALT), the largest component of the lymphoid organ system, is a principal site of both virus production and depletion of primarily lamina propria memory CD4+ T cells. CD4-expressing T cells that previously encountered antigens and microbes are homed to the lamina propria of GALT [2]. The scale of this CD4+ T-cell depletion has adverse effects on the immune system of the host, emphasizing the significance of developing countermeasures to SIV that are effective before infection of GALT. The SIV directly killed a massive number of immune cells in the gut within days of infection. The gut-associated lymphoid tissue (GALT) is an important early site for HIV replication and severe $CD4^+$ T cell depletion [3]. Viral suppression and immune restoration exists in the gastrointestinal mucosa of human immunodeficiency virus type 1-infected patients initiating therapy during primary or chronic infection [3]. In individuals treated with highly active anti-retroviral therapy (HAART), the plasma HIV RNA is reduced to below the level of detection, but there is strong evidence of continuing viral replication after suppression of plasma viremia. It is apparent that a viral reservoir persists in virtually all infected individuals receiving HAART [4,5]. As shown previously, HIV-1 was also detected in bowel crypt cells and the lamina propria in HIV-positive patients [6]. Since these cells are in close contact with intestinal bacteria, it may be possible that bowel bacteria are involved in the pathogenesis of the disease. These findings, confirming that the gut and other mucosal tissue, rather than blood, is the major site of HIV infection and $CD4^+$T cell loss, suggest the possibility that bacteria might serve as a viral reservoir as well.

Based on our previous results concerning detection of HIV-1 sequences in gastrointestinal tracts of HIV-positive patients [7–9], we analysed bacteria and yeasts isolated from the respiratory tract (nose, pharyngeal swabs) of Cambodian and Kenyan HIV-positive children for presence of HIV-like sequences. The study protocol was reviewed and approved by the *Ethics Committee of St. Elizabeth University*.

Material and Methods

DNA isolation and PCR amplification

Bacteria and yeasts from the respiratory tract (nose, pharyngeal swabs) were collected from 39 Cambodian and 28 Kenyan HIV-positive children.

Collected samples were directly transported from Cambodia and Kenya to the Cancer Research Institute in Bratislava and incubated overnight in LB medium at 37°C for amplification. Bacterial chromosomal DNA was prepared by standard protocol [10,11] and by Qiagen kit (Qiagen). Plasmid DNA was purified by an alkaline lysis procedure (Table 1).

To avoid false positive results, 3 PCR reactions were performed as arbitrary controls in every set of reactions. The PCR products, used for sequencing and for HIV-specific probe, were purified through LMP agarose and by QIAquick PCR purification kit (Qiagen). Plasmid pBH10 (genebank accession number M15654) was used as a reference source of HIV DNA and lymphocytes' DNA of HIV/AIDS patient 30 was used as a template for PCR products.

Dot-blot hybridization

For dot-blotting, bacteria and yeasts isolated from patients were amplified overnight in LB medium, and chromosomal DNA was prepared. DNA (0.25 µg/sample) of each patient was transferred to Hybond N^+ membrane, lysed and prehybridized. For probe, the 3 aforementioned purified PCR products were synthesized on DNA template: a) HIV/AIDS patient 30; b) plasmid pBH10. ^{32}P-labelled probe was obtained by Ready-To-Go DNA Labelling Kit (Amersham Bioscience, England). Hybridization was performed for 16 hours in standard hybridization buffer at 42°C or in Rapid-hyb buffer (Amersham Bioscience) at 60°C, and washing was carried out as described previously [9]. To exclude potential contamination, 8 DNA samples of healthy persons were analysed together with tested children.

Southern hybridization

For colony blotting, bacterial suspension was diluted to the concentration of 10^{-10} or 10^{-9} on LB plates and grown colonies were blotted to the Hybond N+ membrane, lysed, washed and prehybridized. ^{32}P-labeled probes were obtained using Ready-To-Go DNA Labeling kit (-dCTP) (Amersham Bioscience). The combined PCR probe was prepared as a mixture of all 3 aforementioned PCR products. Hybridization was performed for 16

Table 1. The PCR specific for HIV sequences was carried out using the following primers.

Gene	Primer	Sequences 5′-3′	Product size (bp)	Position
gag	38for	ATAATCCACCTATCCCAGTAGGAGAAT	115	1075
	39rev	TTTGGTCCTTGTCTTATGTCCAGAATG		1162
pol	Pfor	CATTTGGAAAGGACCAGCAAAACTACT	1484	4457
	Erev	TCATATGCTTTAGCATCTGATGCACAA		5914
env	68for	AGCAGCAGGAAGCACTATGG	142	7302
	69rev	CCAGACTGTGAGTTGCAACAG		7423

Table 2. Distribution of bacteria isolated from Cambodian (A)/Kenyan (B) HIV positive children and summary of hybridization results with HIV-1 specific probes.

A. 39 Cambodian HIV positive children.

Bacterial strain	Number	Positive hybridization
Klebsiella pneumoniae	12 (31.0%)	6 (50.0%)
Candida albicans	5 (13.0%)	3 (60.0%)
Staphylococcus aureus	15 (38.5%)	2 (13.5%)
(MRSA)	2 (5.0%)	1 (50.0%)
Streptococcus pyogenes	3 (8.0%)	2 (67.0%)
Escherichia coli	1 (2.5%)	1 (100.0%)
Proteus mirabilis	1 (2.5%)	1 (100.0%)
	39	16 (41.0%)

B. 28 Kenyan HIV positive children.

Bacterial strain	Number	Positive hybridization
Klebsiella pneumoniae	6 (21%)	4 (67%)
Candida albicans	7 (25%)	0 (0%)
Staphylococcus aureus	11 (39%)	1 (10%)
(MRSA)	1 (4%)	1 (100%)
Proteus mirabilis	2 (7%)	2 (100%)
Candida tropicalis	1 (4%)	1 (100%)
	28	8 (29%)

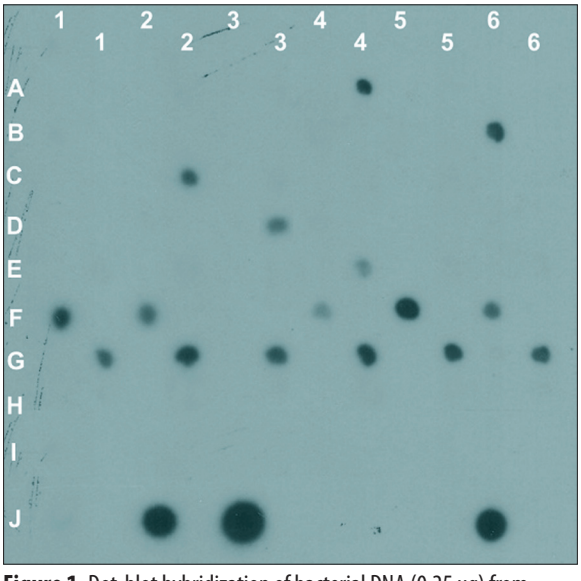

Figure 1. Dot-blot hybridization of bacterial DNA (0.25 µg) from Cambodian HIV positive children. The hybridization probe was mixture of purified PCR products that represented *gag*, *pol* and *env* HIV-1 genes synthesized on the template of plasmid pHB10. Samples of 39 patients were applied in lines from A to G. The control samples of 8 healthy persons are located in lines H and I. In the last line J in position 6 is DNA of tested child with shining clinically expression of disease and in positions 2, 3 are mixtures of aforementioned PCR products in dilution 1:100 and 1:50.

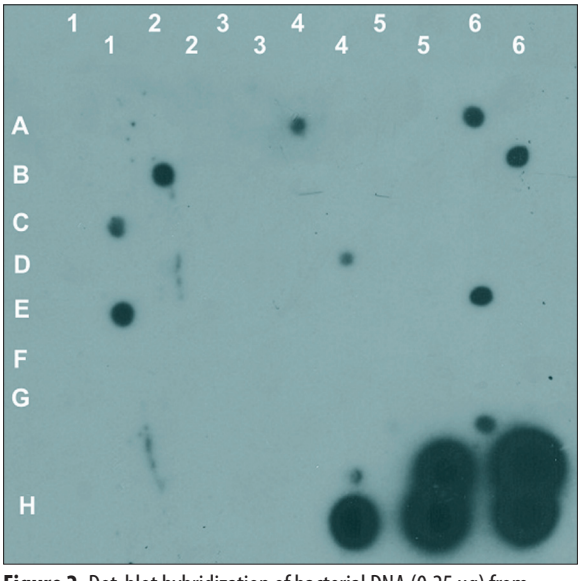

Figure 2. Dot-blot hybridization of bacterial DNA (0.25 µg) from Kenyan HIV positive children. The hybridization probe was mixture of purified PCR products represented *gag*, *pol* and *env* HIV-1 genes synthesized on template of patient 30. Samples of 28 patients were applied in lines from A to E. The control samples of 8 healthy persons are located in lines F and G. In the last line H in position 4 is DNA of the child with shining clinically expression of disease and in positions 5, 6 are mixtures of aforementioned PCR products in dilution 1:100 and 1:50.

hours in standard hybridization buffer at 42°C or in Rapid-hyb buffer (Amersham Bioscience) at 60°C. Subsequently, membranes were washed at final temperature 60°C, resp. 65°C.

DNA sequencing

The PCR products determined by primers 68, 69 synthesized on the template of bacterial DNA were directly sequenced on the ABI 373 DNA Sequencer and ABI PRISM 310 Genetic Analyzer (Applied Biosystem). The sequencing reaction was performed using fluorescent dyes of the ABI Prism Big Dye Terminator sequencing kit (Applied Biosystems) and afterwards extension products were purified by Auto-Seq G-50 columns (Amersham Biosciences).

Results

The bacteria and yeast isolates from the respiratory tract of Cambodian and Kenyan HIV-positive children were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella*

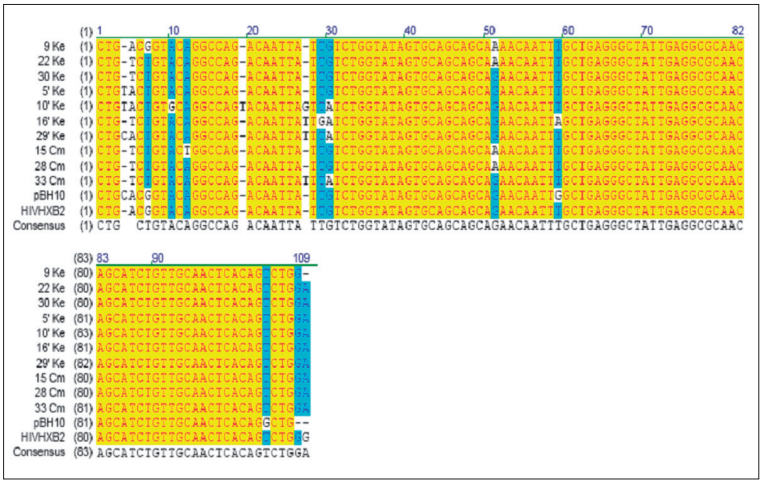

Figure 3. Comparison of the sequences of 142 bp PCR products synthesized on the template of Kenyan (Ke) and Cambodian (Cm) patient bacterial DNA, determined by primers 68;69, of patients: 9 Ke, 22 Ke, 30 Ke, 5'Ke, 10'Ke, 16'Ke, 29'Ke, 15 Cm, Cm.28, 33 Cm. As a reference sequences was used HIV-1 isolate HIV/HXB2.

pneumoniae, *Escherichia coli*, *Proteus mirabilis*, *Candida albicans* and *Candida tropicalis* (Table 2). There were no evident differences between the proportions of species from children of both countries; however, differences were detected in dot-blot hybridization with HIV-1 probes (Figures 1, 2). DNA isolated from 39 tested Cambodian children hybridized for 41% (16 samples) with used probes and for 29% (8 samples) from 28 tested Kenyan children. The most frequently found bacteria in both Cambodian [12] and Kenyan [6] children was *Klebsiella pneumoniae*. This bacteria was positive in hybridization for 50% and 67% (Table 2). *Candida albicans* of 7 Kenyan patients was completely negative in colony and dot-blot hybridization, but was positive in 3 out of 5 Cambodian children. *Escherichia coli* detected in 3 Cambodian children hybridized positively in 2 cases. *Candida tropicalis* was found in 1 patient from Kenya and hybridized with HIV-1 probes. *Proteus mirabilis* of 1 Cambodian child was found positive in hybridization with used probes.

The results of colony (data not shown) and dot-blot hybridization were highly compatible. The differences in hybridization using probes of PCR products synthesized on 2 different templates – pBH10 and DNA of patient 30 – were not significant.

The 142 bp amplicon limited by primers 68; 69 produced using template DNA from both bacteria and lymphocytes of Kenyan (9 Ke, 22 Ke, 10' Ke, 30 Ke, 5' Ke, 16' Ke, 29 Ke) and Cambodian children (15 Cm, 28 Cm, 33 Cm) was sequenced (Figure 3). Amplicons from these different sources were more than 90% identical with reference sequences (HIV/HXB2). Some differences were found in the first part of sequenced fragments. Largest differences were detected between the isolates as a group and the reference sequence in pBH10.

Discussion

Recent observations suggest that the main fight against the HIV disease process is performed in gut-associated lymphatic tissue closed to the gastrointestinal tract [1–3,11]. Our understanding about the restoration of the gut mucosal immune system during highly active antiretroviral therapy is very limited. A dramatic loss of $CD4^+$T cells, predominantly from the mucosal surfaces, suggests the question of whether bacteria play some role in this process. Our previous detection of HIV-like sequences in gut bacteria of HIV/AIDS patients may confirm that bacteria are involved in this trial [7,12,13]. Accordingly, in the respiratory tract bacteria of HIV-positive children from Cambodia and Kenya, HIV-like sequences were detected in 41% and 29%, respectively, of samples. *Klebsiella pneumoniae*, detected most frequently in both cohorts, hybridized with HIV-1-specific probes in 50% and 67%, respectively. These results were expected, because Klebsiella is a gut commensal localized in the intestinal tract, where previously detected bacteria bearing HIV-like sequences were found [12]. The second most isolated *Staphylococcus aureus*, colonizing mostly skin and/or respiratory tract, hybridized only 13.5% and 10%, respectively, with HIV-1-specific probes. *Candida tropicalis* was detected once, with a positive hybridization signal.

On the basis of our previous detection of HIV-like sequences in bacteria isolated from the respiratory tract of AIDS patients [7], it is possible to conclude that bacteria bearing HIV-like sequences are localized not only in the intestinal tract of HIV/AIDS patients, but in other organs as well [12,13]. The transmission of these organisms and their role in AIDS pathogenesis is not clear. Some bacteria probably may serve as a reservoir of HIV-like sequences in the form of "virus-like HIV particles" or as HIV. Our sequencing results showed a very high homology (over 90%) between bacterial HIV-like sequences and HIV-1 isolate HXB2 (HIVHXB2CG). Because all the above mentioned species are gut or skin commensals that cannot be eliminated, they may represent continual imminency for the host.

On the other hand, differences in homology of patient's *env* sequences limited by primers 68;69 with corresponding pBH10 sequences, eliminated to a large extent suspicion of contamination. HIV-1 sequences presented in pBH10 are only one source of retroviral genetic information in laboratory.

There is increasing evidence that the mucosa-associated bacteria may play important roles in the pathogenesis of inflammatory bowel disease, ulcerative colitis, Crohn's disease and potentially even colon cancer [14,15]. Invasive strains of *E. coli* that undergo lyses upon entry into mammalian cells can act as a stable DNA delivery system to their hosts [16]. They

work on the basis of "hit and run away", and their extrachromosomal content remains mainly in the host cell, even when the bacterial carriers are not detectable. Horizontal gene transfer from bacteria to yeast, to plant and mammalian cells has been reported by other investigators [16–19].

CONCLUSIONS

Bacteria and yeasts from the respiratory tract of 41% of Cambodian and 31% of Kenyan HIV-positive children bear HIV-like sequences. According to our preliminary results, we conclude that the ability of invasive bacteria containing HIV sequences in the form of "virus-like particles" to enter into HL-60 cells or human lymphocytes represents an ideal system for of horizontal transfer of genes between eukaryotic and prokaryotic cells. In this way "virus-like particles" or other particles are introduced into cells of the lymphoproliferative system, and, consequently, their genetic information may interact with or be integrated into the human DNA and induce the HIV disease process [7,8].

Acknowledgements

We are very grateful to S. Ciernikova for her assistance in the preparation of the manuscript.

REFERENCES:

1. Mattapallil JJ, Daniel C, Douek DC et al: Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature, 2005; 434: 1093–97
2. Li QS, Duan LJ, Estes JD et al: Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature, 2005; 434: 1148–52
3. Guadalupe M, Sankaran S, George MD et al: Viral suppression and immune restoration in the gastrointestinal mucosa of human immunodeficiency virus type1-infected patients initiating therapy during primary or chronic infection. J Virol, 2006; 80: 8236–47
4. Chun TW, Davey RT, Ostrowski M et al: Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. Nature Med, 2000; 6: 757–61
5. Siliciano JD, Kajdas J, Finzi D et al: Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nature Med, 2003; 9: 727–28
6. Nelson JA, Wiley CA, Reynolds-Kohler C et al: HIV detected in bowel epithelium from patients with gastrointestinal symptoms. Lancet, 1988; 6: 259–62
7. Zajac V, Kovac M, Ciernikova S et al: Detection of HIV sequences in colon bacteria of AIDS positive patients. Clin Microbiol Infec Suppl, 2005; 11: 53
8. Zajac V, Mego M, Martinicky D et al: Testing of bacteria isolated from HIV/AIDS patients in experimental models. Neuroendocrinology Letters, 2006; 27: 101–4
9. Zajac V, Stevurkova V, Matelova L, Ujhazy E: Detection of HIV-1 sequences in intestinal bacteria of HIV/AIDS patients. Neuroendocrinology Letters, 2007; 28: 591–95
10. Wilson KH, Blitchington RB, Greene RC: Amplification of bacterial 16S Ribosomal DNA with polymerase chain reaction. J Clin Microbiol, 1990; 28: 1942–46
11. Maniatis T, Frisch EF, Sambrook J: Molecular cloning a laboratory manual. Cold Spring Harbor Laboratory, 1982
12. Zajac V, Kirchhoff T, Levy ER et al: Characterisation of an X;17(q12;p13) translocation breakpoint in a female patient with hypomelanosis of Ito and choroid plexus papilloma. Eur J Hum Genet, 1997; 5: 61–68
13. Veazey RS, Lackner AA: HIV swiftly guts the immune system. Nature Med, 2005; 11: 469–70
14. Martin MH, Campbell BJ, Hart CA et al: Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. Gastroenterology, 2004; 127: 80–93
15. Darfeuille-Michaud A, Boudeau J, Bulois P et al: High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. Gastroenterology, 2004; 127: 412–21
16. Heinemann JA, Sprague G.f. Jr: Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. Nature, 1989; 340: 205–9
17. Zambryski PC: Chronicles from Agrobacterium – plant cell DNA transfer story. Annu Rev Plant Physiol Plant Mol Biol, 1992; 43: 465–90
18. Walters VL: Conjugation between bacterial and mammalian cells. Nature Genetics, 2001; 29: 375–76
19. Grillot-Courvalin C, Goussard S, Huetz F et al: Functional gene transfer from intracellular bacteria to mammalian cells. Nature Biotechnology, 1998; 16: 862–66