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

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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

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**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  | ATAATCCACCATCCAGTAGGAGAAT | 115 | 1075 |
|      | 39rev  | TTTGGTCTTGTCTTAGCTCAGATG | 1162 | |
| pol  | Pfor   | CATTGGAAGGAGCAGCAAAACTCT | 1484 | 4457 |
|      | Erev   | TCATATGCTTACAGCTAGCTGACAA | 5914 | |
| env  | 68for  | AGCACGAGAAGGACATATG | 142 | 7302 |
|      | 69rev  | CCAGACTCTGAGTGGCAACAG | 7423 | |
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 pneumoniae, Candida albicans, Staphylococcus aureus (MRSA), and Escherichia coli.

| Bacterial strain          | Number | Positive hybridization |
|---------------------------|--------|------------------------|
| 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%)      |
| **Total**                 | 39     | 16 (41.0%)             |

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

| Bacterial strain          | Number | Positive hybridization |
|---------------------------|--------|------------------------|
| 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%)      |
| **Total**                 | **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 pH10. 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 that represented gag, pol and env HIV-1 genes synthesized on the 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.
**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 (HIV/HXB2CG). 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 pH10 sequences, eliminated to a large extent suspicion of contamination. HIV-1 sequences presented in pH10 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].
work on the basis of “hit and run away”, and their extra-
chromosomal 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 mamma-
lian 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 horizontal transfer of genes between eukary-
otic and prokaryotic cells. In this way “virus-like particles”
or other particles are introduced into cells of the lympho-
proliferative system, and, consequently, their genetic infor-
mation 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.

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