ORIGINAL ARTICLE

Detection of protein homologugs with HIV-1 antigens in bacteria of positive patients – phase II

Katarina Hainova [1], Zuzana Adamcikova [1], Sona Ciernikova [1], Viola Stevurkova [1], Vladimir Krcmery [2], Vladimir Zajac [1,2]

1 Department of Cancer Genetics, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia
2 Saint Elizabeth University of Health, Bratislava, Slovakia

Correspondence to: Assoc. Prof. Vladimir Zajac, PhD.
Cancer Research Institute, Slovak Academy of Sciences,
Vlarska 7, 833 91 Bratislava, Slovakia.
TEL: +421-2-59327318; FAX: +421-2-59327250; E-MAIL: vladimir.zajac@savba.sk

Submitted: 2013-10-21 Accepted: 2013-12-05 Published online: 2014-05-05

Key words: **respiratory and intestinal tract bacteria; microflora of HIV/AIDS positive patients; HIV-1 antigens; Western blott analysis**

Neuroendocrinol Lett 2014; **35**(2):110–115 **PMID:** 24878974 NEL350214A04

Abstract

OBJECTIVES: Human immunodeficiency virus type 1 is widely accepted as the cause of AIDS (Acquires Immunodeficiency Syndrome) but it is necessary to consider other factors, not only HIV, which may be involved in AIDS process. It is apparent that a viral reservoir persists in virtually all infected individuals receiving HAART. Reservoirs were detected in macrophages and other cells of the blood system, in which even very effective HAART was not able to eliminate the virus. Over the last period of time AIDS research has been focused on the gut and other mucosal tissue as the major site of HIV infection and CD4+ T cells loss. Intestinal bacteria and cells associated with GIT are in close vicinity and so has been raised the idea that bacteria may be involved in AIDS pathogenesis.

MATHERIAL/METHODS: Bacteria and yeast isolated from a cohort of 67 Cambodian and Kenyan HIV positive children and from a cohort of 62 Slovak and American AIDS patients were analyzed for detection of expression of HIV-1 antigens p17, p24, p55, gp41 and gp120 (Abcam, UK).

RESULTS: By monoclonal antibodies against HIV-1 proteins p17 and p55 was detected protein with molecular weight of 45–55 kDa. In samples of Cambodian and Kenyan HIV positive children was found 35 kDa protein using MAb against HIV-specific protein p17. By using MAbs against p24 was found protein of 55–60 kDa in Cambodian and Kenyan samples but, suprisingly, no proteins were detected in bacterial extracts of American and Slovak AIDS patients by this MAbs. Using monoclonal antibodies against HIV-1 specific protein gp41 was positive signal identified in 30–35% of samples from both cohorts of patients from Kenya and Cambodia and in 75% of samples from American and Slovak patients. The protein of about 75–85 kDa was detected by MAbs against gp120 only in protein extracts obtained from yeasts *Candida sp*. of Cambodian and Kenyan HIV positive children.

CONCLUSION: The molecular weight of 55 kDa protein was detected by MAbs anti HIV p24, p17+p55. Its molecular weight is comparable to gag-encoded Pr55Gag precursor. Surprisingly, such proteins were not found in bacterial extract from samples of American and Slovak patients by using the MAbs against HIV-specific protein p24. The protein of about 75–85 kDa was detected only in *Candida species*

protein extracts of Cambodian and Kenyan HIV positive children by the MAbs against gp120. In Slovak and American samples, protein reacting with MAbs anti gp120 was not found. These results suggest that there are specific differences between Slovak and American HIV positive patients bacterial proteins on one side and Cambodian and Kenyan on the other. These differences may suggest a diverse bacterial evolution in various geographical areas.

INTRODUCTION

In 1981 was first described AIDS (Acquired immunodeficiency Syndrome) caused by Human immunodeficiency virus type I (HIV-1) and Human immunodeficiency virus type II (HIV-2). The assays for identifying recent HIV infections at the population level are based on the alteration of serological parameters, as well as the newest method based on an increase of HIV genetic diversity with the progress of infection (Smoleń-Dzirba & Wasik 2011). Plasma HIV RNA is dramatically reduced in HIV/AIDS patients treated with highly active antiretroviral therapy (HAART), but residual viral replication is detected after suppression of plasma viremia (Chun *et al.* 2000; Cusini *et al.* 2004). It has also been expressly proven that various forms of HIV reservoirs persist in practically all patients receiving HAART (Finzi *et al.* 1997; Siliciano *et al.* 2003).

HIV persists in peripheral blood mononuclear cells despite sustained, undetectable plasma viraemia resulting from long-term antiretroviral therapy (Veazey *et al.* 1998; Brenchley *et al.* 2004). Over half of the CD4+ T cells in the gut mucosa are lost within the first few weeks after HIV-1 infection and remain consistently low, compared to peripheral blood sources, despite long term antiretroviral therapy; furthermore, of the few CD4+ T cells that persist in the gut, a significant increase in immune activation is observed (Mehandru *et al.* 2006).

Microbial flora is able to overcome intestinal barrier and gain the sytemic circulation in case of advanced HIV infection, when the homeostatic balance between gastrointestinal indigenous bacteria and gut immunity fails. Antiretroviral therapy is not able fully controlled the microbial translocation and there is an inefficient CD4+ reconstitution (Merlini *et al.* 2011).

HIV infection is associated with dramatic damage to the gastrointestinal (GI) tract, including substantial disruption of gut microbiota composition with presence of microbes at higher pathogenic potential compared to less aggressive indigenous organisms, massive loss of gut-residing CD4+ T-cells, and down-regulation of GI tract genes expression (Veazey & Lackner 2005; Brenchley *et al.* 2006; Gori *et al.* 2007; Douek *et al.* 2009). HIV-1 was also detected in bowel crypt cells and the lamina propria in HIV-positive patients (Nelson *et al.* 1988).

These recent findings support the idea that the mucosal and intestine immune system is the major site of viral replication, persistence and CD4+ T cells loss in HIV-1 infected persons (Guadalupe *et al.* 2006; Dandekar 2007; Ling *et al.* 2007; Lackner *et al.* 2009; Ling *et al.* 2010).

Gut associated lymphatic tissue (GALT) cells are in close contact with intestinal microflora, so it is possible, that bowel bacteria are involved in the pathogenesis of the disease. Consequences of the anatomo-functional gastrointestinal barrier breach occurs the progressive failure of mucosal immunity and leakage into the systemic circulation of bacterial by-products, such as lipopolysaccharide (LPS) and bacterial DNA fragments, which contribute to systemic immune activation (Brenchley *et al.* 2006; Jiang *et al.* 2009; Estes *et al.* 2010; Ferri *et al.* 2010). Highly active antiretroviral therapy (HAART) only partially amends gastrointestinal tract anatomo-functional damage (Brenchley *et al.* 2006; Marchetti *et al.* 2010) and intestinal microbiota, further hampering intestinal homeostasis (Hooper *et al.* 2010) and sustaining microbial translocation (Brenchley *et al.* 2006; Paiardini *et al.* 2008). Thus, although circulating microbial products have been shown to decrease during HAART, they remain elevated, in turn affecting immune restoration (Brenchley *et al.* 2006; Jiang *et al.* 2009; Marchetti *et al.* 2010). Augustin *et al.* determined, that HIV-infected children on HAART who received any antibiotic were significantly more colonized by clotrimoxazole-resistant *E. coli* ($p<0.01$) than those not receiving any antibiotic prior to colonization (Augustin *et al.* 2008).

In untreated HIV/AIDS, the highest degree of microbial translocation has been shown in patients with severe immune depression (Brenchley *et al.* 2006; Jiang *et al.* 2009). Similarly, following HAART initiation, patients with blunted long-term $CD4^+$ recovery show persistently elevated circulating LPS and bacterial DNA independently of HIV viremia reduction (Brenchley *et al.* 2006; Marchetti *et al.* 2008; Jiang *et al.* 2009).

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 bearing HIV-like sequences (Zajac *et al.* 2007; Zajac *et al.* 2011) might play very important role in AIDS process and serve as a viral reservoir as well. Study of the expression of these HIV-like sequences may confirm this presumption.

MATERIAL AND METHODS

Bacteria and yeasts isolated from a cohort of 67 Cambodian and Kenyan HIV positive children of the respiratory tract (nose, pharyngeal swabs) and from a cohort of 62 Slovak and American AIDS patients (intestinal tract) were used for preparation of protein extracts. The preparated extracts were analyzed for detection of expression of HIV-1 antigens by Western blottnig.

HIV-1 antigens p17, p24, p55, gp41 and gp120 (Abcam, UK) were detected. Isolated proteins after overnight at 45 mA electrophoresis were transferred from acrylamide gel to nitrocellulose (NC Hybon membrane). The membrane was incubated in TBS-T buffer and blocked using 5% milk for 1 hour at room temperature. The blocking buffer was removed and the membrane was washed with TBS-T buffer. Appropriately diluted monoclonal mouse antibodies p17, p24, p55, gp41 and gp120 (Abcam, UK) in TBS-T buffer with 5% milk was added to the membrane and incubated overnight at 4 °C on a shaker with a rocking motion. The membrane was washed with TBS-T buffer and incubated for 1 hour at room temperature in appropriately diluted goat anti-mouse antibody SC-2005 (Santa Cruz Biotech) in buffer containing 5% milk. Consequently the membrane was washed with TBS-T buffer and for visualization ECL solutions were used.

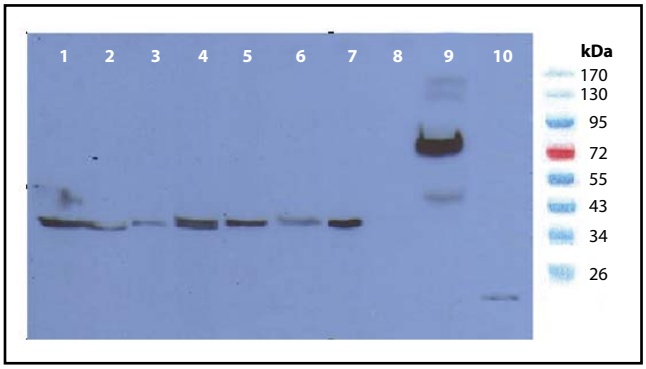

Fig. 1. Western blotting of proteins isolated from bacteria and yeasts of the respiratory tract (nose, pharyngeal swabs) of Cambodian (Km) and Kenyan (Ke) HIV positive children. Detection was performed using monoclonal antibodies against HIV-1 p17 diluted 1:750. Line 1: 3´Km, line 2: 10 Km, line 3: 14´Ke, line 4: 17´Ke, line 5: 21 Ke, line 6: 28 Km, line 7: 14´Km, line 8: negative bacterial control, line 9: serum of AIDS patient diluted 1:500, line 10: protein p17 diluted 1:750.

RESULTS

Microbes of Slovak and American HIV/AIDS patients were most often identified as *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Citrobacter freundii* and *Enterobacter aerogenes.* Microbes from respiratory tract of Cambodian and Kenyan HIV/AIDS patients were most often identified as *Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans, Candida tropicalis, Enterobacter aerogenes,* but also as *Escherichia coli* and *Proteus mirabilis.* Taxonomic classification of analyzed microbes was performed in comercial diagnostic laboratory using standard microbiological tests.

Protein of 35 kDa was also detected in bacteria and yeasts of the respiratory tract of Cambodian (Km) and Kenyan (Ke) HIV positive children: 3´Km, 10 Km, 14´Ke, 17´Ke, 21 Ke, 28 Km, 14´Km, using MAbs against HIV1 p17 (Figure 1).

In our research was detected 55–60 kDa protein using MAbs against HIV1 p24 approximately in 30 Cambodian (Km) and 35% Kenyan (Ke) bacterial extracts of HIV positive patients: 14´Ke, 17´Ke, 21 Ke, 32'Ke, 3´Km, 14'Km, Muta 104-0 (Figure 2).

45–55 kDa protein was also detected in bacteria isolated from intestinal tract Slovak and American HIV positive pacients: 132/3, 18315, Tu Sevcikova 3, Tu S6, Bact 28/38 II, 883 Ca^{+}/2, 18363, serum of AIDS patient diluted 1:500, 21 Ke using MAbs against HIV-1 p55+p17 (Figure 3).

Protein of 41 kDa was also detected in bacteria isolated from intestinal tract Slovak and American HIV positive pacients: Mok1, P8, 79, P55 + IPTG, K1-1, 116x, serum of AIDS patient diluted 1:500 using MAbs against HIV-1 gp41 (Figure 4).

Using monoclonal antibodies against HIV-1 gp120 (1:750), proteins of 75–85 kDa were detected in protein extracts from bacteria and yeasts of the respiratory tract (nose, pharyngeal swabs) of Cambodian (Km) and

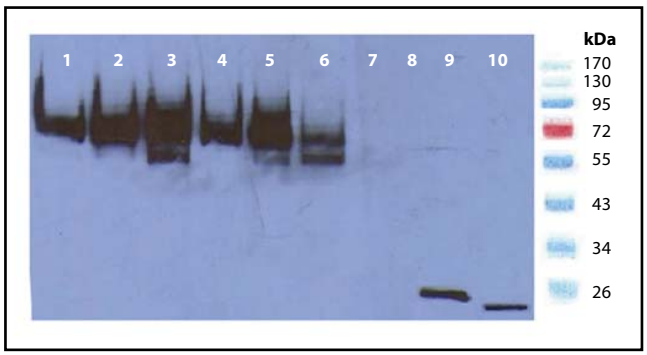

Fig. 2. Western blotting of proteins isolated from bacteria and yeasts of the respiratory tract of Cambodian (Km) and Kenyan (Ke) HIV positive children. Used monoclonal antibodies against HIV-1 p24 (1:1000). Line 1: 14´Ke, line 2: 17´Ke, line 3: 21 Ke, line 4: 32´Ke, line 5: 3´Km, line 6: 14´Km, line 7: Muta 104-0, line 8: negative bacterial control, line 9: serum of AIDS patient diluted 1:500, line 10: protein p24 diluted 1:1000.

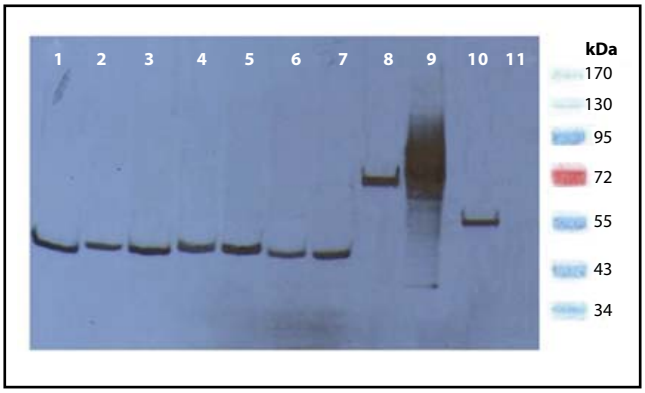

Fig. 3. Western blotting of proteins isolated from intestinal bacteria of Slovak and American HIV positive patients. Used monoclonal antibodies against HIV-1 p55+p17 (1:1000). Line 1: 132/3, line 2: 18315, line 3: Tu Sevcikova 3, line 4: Tu S6, line 5: Bact 28/38 II, line 6: 883 Ca^{+}/2, line 7: 18363, line 8: serum of AIDS patient diluted 1:500 , line 9: 21 Ke, line 10: protein p55 diluted 1:1000, line 11: negative bacterial control.

Kenyan (Ke) HIV positive children: 14 Ke, 21 Ke, 3'Km, 22 Km, 28 Km, 25´Km, serum of AIDS patient diluted 1:500 (Figure 5).

DISCUSSION

Recent studies suggest that the main fight against the HIV diseases is performed in gut-associated lymphatic tissue closed to the gastrointestinal tract (Li *et al.* 2005; Guadalupe *et al.* 2006; Mach *et al.* 2007). Severely immune depressed HIV-infected patients fail to efficiently control translocation of microbial macromolecules following HAART initiation and maintaining heightened microbial translocation on virologically-suppressive HAART display a circulating microbiota that is polymicrobic at the genotype level and that is not substantially modified by therapy (Merlini *et al.* 2011). It is questionable if bacteria play some role in process of dramatic loss of $CD4^+$ T cells, predominantly from the mucosal surfaces. Less efficient control over microbial translocation in HIV patients' cohort might be consistent with the dramatic structural damage of the intestinal barrier described in untreated advanced HIV/SIV infection (Brenchley *et al.* 2006; Estes *et al.* 2008), with ever-increasing content of microbial by-products infiltrating intestinal lamina propria and gaining the circulation. The gastrointestinal tract damage and occurring microbial translocation might be hardly repaired by the late institution of therapy, in turn favouring the continuous passage into the systemic circulation of a highly polymicrobic intestinal flora. Increased levels of circulating microbial by-products and markers of immune response to microbial translocation have been associated with increased HIV disease progression and mortality even in the context of continuous antiviral therapy and independently of $CD4^+$ count and HIV-viremia (Marchetti *et al.* 2010; Sandler *et al.* 2011). Interestingly, more than 90% of HIV-infected patients harbour a bacterial population enriched with *Enterobacteriales*, whereas less than 60% display the probiotic *Lactobacilllales*, with the same proportion being maintained on virologically-suppressive HAART (Brenchley *et al.* 2006).

Previous studies in our laboratory detected HIV-like sequences in gut bacteria of HIV/AIDS patients, which may confirm that bacteria could be involved in this trial (Veazey & Lackner 2005; Zajac *et al.* 2007; Zajac *et al.* 2011).

In summary, the molecular weight of 45–55 kDa protein was detected by MAbs anti HIV-1 p17 and p55. Its molecular weight is comparable to *gag*-encoded Pr55Gag precursor. This precursor like protein of 55–60 kDa was detected by MAbs against HIV p24 in Cambodian and Kenyan samples. Surprisingly, no such proteins were found in bacterial extract of Slovak and American AIDS patients by this MAbs. Using MAbs against gp41 the protein of 41 kDa was identified in 30–35% of bacterial extracts from all cohorts of patients. The protein of about 75–85 kDa was detected only in *Candida* species protein extracts of Cambodian and Kenyan HIV positive children by the MAbs against gp120. In Slovak and American samples, protein reacting with MAbs anti gp120 was not found. These results suggest that there are specific differences between Slovak and American HIV positive patients bacterial proteins on one side and Cambodian and Kenyan on the other.

Detection of HIV-1 antigens in bacteria isolated from respiratory and gastrointestinal tract of HIV patients, it is possible to conclude that bacteria bearing HIV-1 specific proteins are localized not only in the intestinal tract of HIV/AIDS patients but in the other organs. Consistent with earlier observation in SIV models, Veazey reminds us that the battle against HIV-1 should focus on the intestinal mucosa with therapeutic strategies to reduce gut immune activation (Veazey & Lackner 2006). As well as systemic immune activation in SIV-infected rhesus monkeys is associated with breakdown of the intestinal epithelial lining (Estes *et al.* 2010;

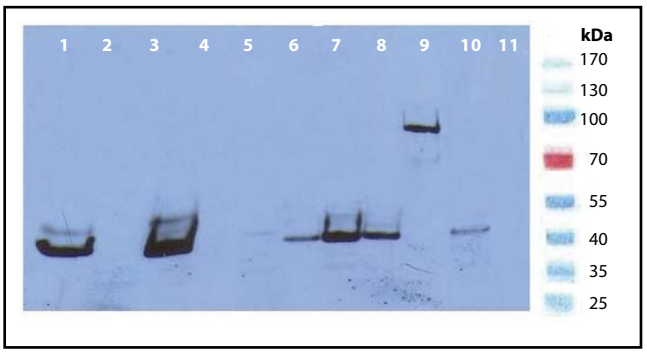

Fig. 4. Western blotting of proteins isolated from intestinal bacteria of Slovak and American HIV positive patients. For detection monoclonal antibodies against HIV-1 gp41 diluted 1:750 were used. Line 1: Mok1, line 2: P8, line 3: 79, line 4: E.coli H, line 5: P14, line 6: P15 + IPTG, line 7: K1-1, line 8: 116x, line 9: serum of AIDS patient diluted 1:500, line 10: protein gp41 diluted 1:500, line 11: negative bacterial control.

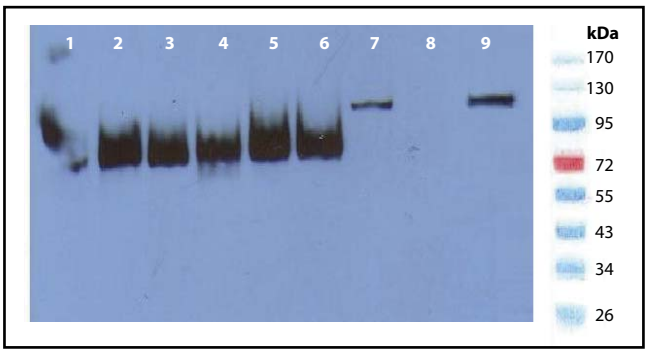

Fig. 5. Western blotting of proteins isolated from bacteria and yeasts of the respiratory tract (nose, pharyngeal swabs) of Cambodian (Km) and Kenyan (Ke) HIV positive children. For detection monoclonal antibodies against HIV-1 gp120 diluted 1:750 were used. Line 1: 14 Ke, line 2: 21 Ke, line 3: 3'Km, line 4: 22 Km, line 5: 28 Km, line 6: 25´Km, line 7: serum of AIDS patient diluted 1:500, line 8: negative bacterial control, line 9: protein gp120 diluted 1:500.

Sandler & Douek 2012). A mechanism contributing to AIDS progression in which intestinal epithelial damage leads to translocation antigens into tissues, which contributes to systemic immune activation, increased lentiviruses replication, progressive immune deficiency and AIDS (Sandler & Douek 2012). Despite the importance of intestinal barrier damage to AIDS progression, the mechanisms responsible for AIDS enteropathy are not understood. Virgin *et al.* said that virome understood as a subset of the metagenome may be defined to include both viruses that infect eukaryotic cells and phages that infect other members of the microbiome. So the mammalian virome and bacterial microbiome are extremly complex and can contribute to immune status and disease in a range of settings (Virgin *et al.* 2009). The enteric virome contributes to the progression of SIV infection to AIDS by fostering intestinal epithelial damage and systematic immune activation via release of pathogens as well as bacterial, fungal antigens into host tissues and systematic circulation (Scott *et al.* 2012).

The transmission of HIV patients´ microorganisms and their role in AIDS pathogenesis is still not explained, but is intensivelly studied. Bacteria bearing HIV sequences may serve as a reservoir of HIV-1 antigens in the form of "virus-like HIV particles" or others extrachromosomal forms. It is likely that virus-like HIV particles does not consist of viral structures, but of cellular proteins and protein particles belonging to bacteria. Such particles could induce some inflammatory signals and probably again the actions attributed to HIV. We believe that in the process of immunodeficiency play important role combination of gastrointestinal microflora and HIV genetic information.

Thus, our research should be used to generate hypotheses to be tested in larger studies aimed at investigating the role of translocating bacteria in gastrointestinal tract. Our study will be extended to the new patients cohort. The Western blott analysis that we used, will be in next accompanied by more precise techniques such as immunoprecipitation and subsequent sequencing of isolated proteins.

ACKNOWLEDGEMENT

The autors would like to thank prof. A. Liskova from St. Elizabeth University College of Health and Social Sciences, Bratislava, Slovakia for Kenyan and Cambodian patients samples and dr. M. Mokras from Department of Infectious and Geografic Medicine Derer´s Hospital Bratislava for providing Slovak patients bacteria. Our thanks go to dr. D. Stanekova from Slovak Medical University for viral load analysis. This work was supported by the grants VEGA 2/0081/08, VEGA 2/0096/11, grant APVV-0404-07 and APPV-06-46-11. This publication is also the result of the project implementation: SF ITMS project code: 26240220058 supported by the Research & Development Operational Programme funded by the ERDF.

REFERENCES

1 Augustin A, Shahum A, Kalavsky E, Liskova A, Kisac P, Krcmery V (2008). Colonization of the respiratory tract by drug-resistant bacteria in HIV-infected children and prior exposure to antimicrobials. Med Sci Monit. **14**(12): SC19–22.
2 Brenchley JM, Price DA, Douek DC (2006). HIV disease: fallout from a mucosal catastrophe? Nat Immunol. **7**: 235–239.
3 Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L,Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. **12**: 1365–1371.
4 Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC (2004). $CCD4^+$ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. Journal exp med. **200**: 749–759.
5 Chun TW, Davey RT, Ostrowski M, Shawn Justement J, Engel D, Mullins JI, Fauci AS (2000). Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. Nature Medicine. **6**: 757–61.
6 Cusini M, Salmaso F, Zerboni R, Carminati G, Vernaci C, Franchi C, Locatelli A, Alessi E (2004). 5% Imiquimod cream for external anogenital warts in HIVinfected patients under HAART therapy. Int J STD AIDS. **15**: 17–20.
7 Dandekar S (2007). Pathogenesis of HIV in the gastrointestinal tract. Curr HIV/AIDS Rep. **4**(1): 10–15.
8 Douek DC, Roederer M, Koup RA (2009). Emerging concepts in the immunopathogenesis of AIDS. Annu Rev Med. **60**: 471–484.
9 Estes J, Baker JV, Brenchley JM, Khoruts A, Barthold JL, Bantle A, Reilly CS, Beilman GJ, George ME, Douek DC, Haase AT, Schacker TW (2008). Collagen deposition limits immune reconstitution in the gut. J Infect Dis. **198**: 456–464.
10 Estes JD, Harris LD, Klatt NR, Tabb B, Pittaluga S, Paiardini M, Barclay GR, Smedley J, Pung R, Oliveira KM, Hirsch VM, Silvestri G, Douek DC, Miller CJ, Haase AT, Lifson J, Brenchley JM (2010). Damaged intestinal epithelial integrity linked to microbial translocation in pathogenic simian immunodeficiency virus infections. PLoS Pathog. **6**(8): e1001052.
11 Ferri E, Novati S, Casiraghi M, Sambri V, Genco F, Gulminetti R, Bandi C (2010). Plasma levels of bacterial DNA in HIV infection: the limits of quantitative polymerase chain reaction. J Infect Dis. **202**: 176–177.
12 Finzi D, Hermankova M, Pierson T, Caruuth LM, Buck Ch, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF (1997). Identification of a reservoir for HIV-1 in patients on Highly Active Antiretroviral Therapy. Science. **278**: 1295–1300.
13 Gori A, Tincati C, Rizzardini G, Torti C, Quirino T, Haarman M, Ben Amor K, van Schaik J, Vriesema A, Knol J, Marchetti G, Welling G, Clerici M (2007). Early Impairment of Gut Function and Gut Flora Supports a Role for the Alteration of the Gastrointestinal Mucosa in HIV Pathogenesis. J Clin Microbiol. **46**(2): 757–8.
14 Guadalupe M, Sankaran S, George D, Reay E, Verhoeven D, Shacklett BL, Flamm J, Wegelin J, Prindiville T, Dandekar S (2006). Viral supression and immune restoration in the gastrointestinal mucosa of humanimmunodeficiency virus type 1-infected patients initiating therapy during primary or chronic infection. J Virol. **80**: 8236–47.
15 Hooper LV, Macpherson AJ (2010). Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol. **10**: 159–169.
16 Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, Landay A, Martin J, Sinclair E, Asher AI, Deeks SG, Douek DC, Brenchley JM (2009). Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis. **199**: 1177–1185.

17 Lackner AA, Mohan M, Veazey RS (2009). The gastrointestinal tract and AIDS pathogenesis. Gastroenterology. **136**(6): 1965–78.
18 Li QS, Duan LJ, Estes JD, Ma ZM, Rourke T, Wang Y, Reilly C, Carlis J, Miller CJ, Haase AT (2005). Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature. **434**: 1148–52.
19 Ling B, Mohan M, Lackner AA, Green LC, Marx PA, Doyle LA, Veazey RS (2010). The large intestine as a major reservoir for simian immunodeficiency virus in macaques with long-term, nonprogressing. J Infect Dis. **202**(12): 1846–54.
20 Ling B, Veazey RS, Hart M, Lackner AA, Kuroda M, Pahar B, Marx PA (2007). Early restoration of mucosal CD4 memory CCR5 T cells in the gut of SIV-infected rhesus predicts long non-progression. AIDS. **21**(18): 2377–85.
21 Mach T, Skwara P, Biesiada G, Cieśla A, Macura A (2007). Morphological changes of the upper gastrointestinal tract mucosa and Helicobacter pylori infection in HIV-positive patients with severe immunodeficiency and symptoms od dyspepsia, Med Sci Monit. **13**(1): CR14–19.
22 Marchetti G, Bellistri GM, Borghi E, Tincati C, Ferramosca S, La Francesca M, Morace G, Gori A, Monforte AD (2008). Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy. Aids. **22**: 2035–2038.
23 Marchetti G, Cozzi-Lepri A, Bellistrı` G, Merlini E, Chiodera A, *et al.* (2010). Role of microbial translocation and immune hyeractivation in disease progression of HIV+ patients with preserved CD4 count in the absence of HAART. February 16–19 2010; San Francisco, CA, USA.
24 Mehandru S, Poles MA, Tenner-Racz K, Jean-Pierre P, Manuelli V, Lopez P, Shet A, Low A, Mohri H, Boden D, Racz P, Markowitz M (2006). Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med. **3**: 484.
25 Merlini E, Bai F, Bellistri GM, Tincati C, d´Arminio Monforte A, Marchetti G (2011) Evidence for polymicrobic flora translocating in peripheral blood of HIV-infected patients with poor immune response to antiretroviral therapy. PloS One. **6**(4): e18580.
26 Nelson JA, Wiley CA, Reynolds-Kohler C, Margaretten W, Wiley CA, Reese ChE, Levy JA (1988). HIV detected in bowel epithelium from patients with gastrointestinal symptoms. Lancet. **6**: 259–62.
27 Paiardini M, Frank I, Pandrea I, Apetrei C, Silvestri G (2008). Mucosal immune dysfunction in AIDS pathogenesis. AIDS Rev. **10**: 36–46.
28 Sandler NG, Douek DC (2012). Microbial translocation in HIV infection: causes, consequences and treatment opportunities. Nat Rev Microbiol. **10**(9): 655–666.
29 Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC (2011). Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection. J Infect Dis. **203**(6): 780-790.
30 Scott A, Handley SA, Thackray LB, Zhao G, Presti R, Miller AD, Droit L, Abbink P, Maxfield LF, Kambal A, Duan E, Stanley K,Kramer J, Macri SC, Permar SR, Schmitz JE, Mansfield K, Brenchley JM, Veazey RS, Stappenbeck TS, Wang D, Barouch DH, Virgin HW (2012). Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. Cell. **151**(2): 253–266.
31 Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 on resting CD4+ T cells. Nature Medicine. **9**: 727–8.
32 Smoleń-Dzirba J, Wasik TJ (2011). Current and future assays for identifying recent HIV infections at the population level. Med Sci Monit. **17**(5): RA124–133.
33 Veazey RS, DeMaria MA, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA (1998). Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science. **280**: 427–31.
34 Veazey RS, Lackner AA (2005). HIV swiftly guts the immune system. Nature Medicine. **11**: 469–70.
35 Veazey RS, Lackner AA (2006). Impact of antiretroviral therapy on intestinal lymphoid tissues in HIV infection. PLoS Med. **3**: 515.
36 Virgin HW, Wherry EJ, Ahmed R (2009). Redefining chronic viral infection. Cell. **138**(1): 30–50.
37 Zajac V, Matelova L, Liskova A, Mego M, Holec V, Adamcikova Z, Stevurkova V, Shahum A, Krcmery V (2011). Confirmation of HIV-like sequences in respiratory tract bacteria of Cambodian and Kenyan HIV-positive pediatric patients. Med Sci Monit. **17**(3): 154–158.
38 Zajac V, Stevurkova V, Matelova L, Ujhazy E (2007). Detection of HIV-1 sequences in intestinal bacteria of HIV/AIDS patients. Neuro Endocrinol Lett. **28**(5): 591–595.