April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 365-374

THE INTESTINAL CMV REPLICATION AND ITS ASSOCIATION WITH EPITHELIAL BARRIER DYSFUNCTION IN HIV-INFECTED INDIVIDUALS

Balaniuk I.V., Moskaliuk V.D., Andrushchak M.O., Sokolenko M.O., Randiuk Yu.O., Boiko Yu.I., Kolotylo T.R.

Bukovinian State Medical University, Chernivtsi, Ukraine

*margaritaassistent@gmail.com

Abstract

Inflammation and dysfunction of the intestinal epithelial barriers are the main signs of HIV-infection that persists despite potent combined ART. The barrier properties of the intestinal mucosal epithelium are supported by a monolayer of cylindrical epithelial cells, which are firmly connected by intercellular dense connections. Weakening of the density of these compounds is the main mechanism for increasing intestinal permeability in patients who have undergone ART. In the context of HIV-infection, the broken integrity of the intestinal epithelial barrier facilitates bacterial translocation, which is the main cause of chronic inflammation in it. It has found that dysfunction of the intestinal epithelial barrier during HIV-infection is due to increased production of inflammatory cytokines by activated mucosal T-cells and epithelial cells that respond directly to gp120 HIV-1. CMV proteins were detected in the cytoplasm of intestinal epithelial cells and leukocytes in all HIV-positive individuals by analyzing recto sigmoid biopsy using IHC and RNA scope ISH methods. All of these people had no symptoms or suspected CMV disease. To determine whether HIV status was associated with an increased incidence of CMV in the gut, an HIV-negative CMV-positive control group was included in the study. CMV proteins in intestinal epithelial cells were not detected in some HIV-negative individuals with CMV infection. The specificity of CMV detection was confirmed by the absence of CMV proteins in intestinal biopsies from HIV-negative CMV-negative people [73]. The results of these studies indicate that CMV-associated violation of the integrity of the epithelium of the colon may be associated to some extent with induced CMV-IL-6. In addition, TNF-α transcripts are expressed in the intestinal mucosa from untreated AIDS patients with CMV colitis and may be associated with macrophage-like cells containing cytomegalovirus inclusions.

Key words: *HIV-infected individuals, Asymptomatic cytomegalovirus coinfection, epithelial cells, cytokine IL-6, antiviral therapy*

Introduction

Asymptomatic cytomegalovirus coinfection (CMV) is almost ubiquitous in HIV-infected individuals. It has recently been hypothesized that sustained CMV replication in the intestinal epithelium of HIV-infected people with the HIV/CMV association impairs intestinal epithelial function. Using the combination of state-of-the-art in situ hybridization technology (RNA scope) and immunohistochemistry, CMV DNA and proteins and the presence of intestinal damage in recto sigmoid specimens in HIV-infected individuals (both untreated and in people receiving antiretroviral therapy) were detected. Two recent different studies together have shown that intestinal epithelial cells fully allow CMV replication [1-4]. Regardless of the presence of HIV-infection in the body, CMV disrupted the close bonds of polarized intestinal cells, significantly reducing trans epithelial electrical stability, monolayer integrity and increased trans epithelial permeability. The effect of CMV infection on the intestinal epithelium is mediated, at least in part, by the CMV-induced proinflammatory cytokine IL-6. Together, the results of several recent studies suggest that CMV can disrupt epithelial connections, leading to dissociation of the intestinal microflora (dysbacteriosis) and chronic inflammation in the intestine. It is possible that CMV may be the target of therapy to prevent or treat dysfunction of the intestinal epithelial barriers and dysbacteriosis during HIV-infection. Finding out the peculiarities of the prevalence of comorbidities in HIV-positive people is a very important issue and has both scientific and practical interest. The majority (78,8%) of HIV-infected patients are diagnosed with concomitant pathology, and most often it is combined. The prevalence of intestinal dysbacteriosis among HIV-infected people is almost 10% higher than the population indicators [5-7]. The exact mechanisms, by which HIV-infection damages the intercellular narrow connections of the intestinal epithelium, remain the relevance research problem.

Methods

The aim of the study was to identify the features of CMV replication in the intestine and the relationship between the presence of CMV and dysfunction of the epithelial barriers in HIV-infected patients.

Results

Inflammation and dysfunction of the intestinal epithelial barriers are the main signs of HIV-infection that persists despite potent combined ART. The barrier properties of the intestinal mucosal epithelium are supported by a monolayer of cylindrical epithelial cells, which are firmly connected by intercellular dense connections. Weakening of the density of these compounds is the main mechanism for increasing intestinal permeability in patients who have undergone ART. In the context of HIV-infection, the broken integrity of the intestinal epithelial barrier facilitates bacterial translocation, which is the main cause of chronic inflammation in it. It has found that dysfunction of the intestinal epithelial barrier during HIV-infection is due to increased production of inflammatory cytokines by activated mucosal T-cells and epithelial cells that respond directly to gp120 HIV-1. [8-11]. However, cellular sources of inflammatory cytokines that lead to intestinal dysfunction in HIV-infected people remain controversial, and the mechanisms described above do not confirm the presence of various opportunistic pathogens, including CMV in the intestine which has been repeatedly proposed as a cofactor in the progression of disease progression, despite suppressive ART. It is important to note that in the gastrointestinal tract there is a serious depletion of CD4 + T cells at all stages of HIV disease, which is not fully restored after ART, which may be the main cause of local and asymptomatic reactivation of CMV. CMV is a common opportunistic pathogen in HIV-infected individuals before ART, leading to significant morbidity and mortality [12-15].

Studies using state-of-the-art dual immunohistochemistry (IHC) and in situ hybridization (ISH) technology have shown that the persistence of CMV in the recto sigmoid tissues of asymptomatic CMV-positive individuals with and without ART has been associated with epithelial dysfunction intestines. The researchers used two different models of the system to study the

availability of intestinal epithelial cells for CMV replication: basic human intestinal cells were differentiated in vitro to form polarized monolayers and a model of a humanized mouse gut. It has been found that, regardless of HIV infection, CMV disrupts the close bonds of polarized intestinal cells, significantly reducing trans epithelial electrical resistance (TER), the integrity of the epithelial monolayer, and increasing the permeability of epithelial barriers. In addition, CMV-associated violation of the integrity of the intestinal epithelium can be caused, at least in part, by CMV-induced proinflammatory cytokine - interleukin-6 (IL-6). It is important to note that Lettermovar, a new anti-CMV drug that is currently in clinical trials, retains epithelial integrity in this system. The results of the studies confirm the potentially active role of CMV in the movement of epithelial barrier dysfunction and microbial translocation. In general, these studies emphasize the possibility of a new method of prevention and treatment of dysfunction of the intestinal epithelial barriers in HIV-infection [16-20].

CMV proteins were detected in the cytoplasm of intestinal epithelial cells and leukocytes in all HIV-positive individuals by analyzing recto sigmoid biopsy using IHC and RNA scope ISH methods. All of these people had no symptoms or suspected CMV disease. To determine whether HIV status was associated with an increased incidence of CMV in the gut, an HIV-negative CMV-positive control group was included in the study. CMV proteins in intestinal epithelial cells were not detected in some HIV-negative individuals with CMV infection. The specificity of CMV detection was confirmed by the absence of CMV proteins in intestinal biopsies from HIV-negative CMV-negative people [21-23].

Assessing whether CMV infection in the intestine is related to violations of epithelial barriers, it should be noted that in recto sigmoid biopsies of HIV/CMV patients, the detection of CMV in intestinal epithelial cells was accompanied by a violation of the continuity of intestinal epithelial connections. Interestingly, the cell connection was unchanged and continuous in the intestinal crypts of HIV-negative CMV-positive people. Although CMV proteins were detected in these biopsies in cells surrounding the intestinal crypts, no CMV proteins were detected in the intestinal epithelial cells and the integrity of the epithelium was not compromised. [25-28]. To confirm the conclusion that the disruption of connections between epithelial cells is specific for CMV, a study of several intestinal biopsies from individuals with clinically diagnosed CMV enteritis and CMV colitis before and after treatment with Val ganciclovir. Numerous cytomegalovirus cells of IE-positive cells were detected in intestinal crypts in all biopsies. Notably, these cytomegalovirus cells often rise above the level of the intestinal monolayer into the lumen, revealing damage to the epithelium. [29].

Discussion

It was found that cytomegalovirus IE-positive epithelial cells are separated into the lumen of the crypt, which has a marked resemblance to the cleavage of cytomegalovirus cells found in the intestine of an individual with active CMV infection. These data indicate that the intestinal epithelium is very susceptible to CMV infection. A model of in vitro polarized cells with primary human colon epithelial cells (HCoEpiC) was invented to recombine CMV infection in recto sigmoid tissues [30]. These cells retain the morphological and functional properties of intestinal epithelial cells during the first four passages. They express cytokeratin, a marker of epithelial cells, and negative to the udder, a mesenchymal cell marker. When CMV was inoculated into HCoEpiC, approximately 30% of the cells expressed CMV proteins on the 1st day, and on the 3rd day, some infected cells expressed CMV glycoprotein in the membrane, indicating a productive infection. When CMV was inoculated into polarized HCoEpiC, only an average of 3.7% of cells expressed CMV proteins on the 1st day. Thus, it was proved that the susceptibility of polarized HCoEpiC to CMV infection is significantly lower than non-polar cells. An intriguing observation of persistently low CMV replication in polarized HCoEpiC may be the basis for the assumption that intestinal epithelial cells, after detachment into the lumen of the intestinal cavity with loss of polarity, create additional target cells for CMV. [31-34]. These data suggest that, although CMV predominantly infects unpolarized intestinal cells, it is able to maintain low levels of replication in highly differentiated polarized intestinal cells. Taken

together, these in vivo and in vitro experiments showed that human intestinal epithelial cells are fully accessible for CMV replication. [35].

During HIV infection, immune activation associated with dysfunction of intestinal epithelial barriers persists despite potent suppressive ART [5-8,36-38]. Its main mechanisms are complex and remain unclear, but the role of opportunistic viral pathogens in the gut has not been fully assessed yet. Independent of HIV, CMV impairs the integrity of polarized human intestinal cells, significantly reducing transepithelial electrical resistance and enhancing the permeability of epithelial barriers. CMV-associated disruption of intestinal epithelial integrity was mediated, at least in part, by CMV-induced IL-6. These observations suggest that CMV reactivation in the gastrointestinal epithelium of HIV-infected individuals may be a potent cofactor that stimulates the release of proinflammatory cytokines from intestinal epithelial cells, weakens mucosal barrier function, and locally initiates bacterial infection and chronic inflammation in the intestines. Cytomegalovirus, as an opportunistic pathogen in HIV-infected individuals [38], has been the cause of significant morbidity and mortality before ART administration [39-41]. In the era of effective ART, CMV remains an important cofactor in the progression of HIV disease [14, 32-36] [20, 21], which indicates a strong link with systemic inflammation [12, 13], the physiology of aging [13, 15, 16], and cardiovascular disease [5, 17-19]. Importantly, in people receiving ART, recovery of T cells in the gastrointestinal tract never reaches the levels observed in uninfected healthy people [6-9] and, thus, can be an important site of CMV reactivation [43]. Active CMV infection is a recognized cause of symptomatic colitis in HIV-infected individuals. The recent studies indicate that CMV also persists in the recto sigmoid tissues of asymptomatic CMV-positive individuals with both untreated and ART-suppressed HIV infection. Both CMV proteins and viral DNA were detected in intestinal epithelial cells in 47% of biopsies in ART-suppressed HIV infection, indicating that intestinal epithelium supports CMV replication. HIV has a well-established cofactor connection with CMV [38]. According to this observation, there is a tendency for an association between the presence of CMV DNA/proteins and HIV RNA in the recto sigmoid tissues of asymptomatic CMV-positive participants with ART-suppressed HIV infection. To determine the role of intestinal epithelial cells in gastrointestinal disease of CMV independently of HIV, their susceptibility to CMV infection was further investigated using the SCID-hu gut model [37-39].

Significant damage to the mucosal epithelium was observed 7 days after inoculation with CMV; infection was expressed by abundant detection of CMV proteins, and the mucous membrane of the damaged intestinal crypts contained numerous IE-positive cytomegalovirus cells, corresponding to those in intestinal biopsy with clinically diagnosed CMV colitis. We are intrigued by the high level of susceptibility of the intestinal epithelium to CMV in vivo and the conclusion that CMV persists in the intestinal epithelium of people co-infected with HIV/ CMV. Although CMV can involve any part of the gastrointestinal tract in AIDS patients, the colon is the most infected site [22-24, 42-44].

Measuring study IL-6 in plasma samples obtained from study of participants immediately prior to biopsy showed a tendency for higher plasma IL-6 levels in individuals in whom recto sigmoid biopsies showed CMV activity compared with intestinal samples from those who have no signs of CMV activity in the intestine [45]. IL-6 is a pleiotropic cytokine that can damage the integrity of the intestinal barrier [46]. CMV is an inducer of TNF-α and IL-1β in the THP-1 monocyte cell line [27, 28] and IL-6 in peripheral blood mononuclear cells, endothelial cells and lung fibroblasts [29-31]. On the other hand, it is well known that the integrity and permeability of barriers at epithelial cell junctions can be compromised by proinflammatory cytokines TNF-α, IL-6 and IL-1β [32-34,47-49]. To investigate whether CMV infection induces the expression of these proinflammatory cytokines in intestinal epithelial cells, IL-6, TNF-α and IL-1β were measured in HCoEpiC culture medium by ELISA. These measurements showed an increase in IL-6 production, but not TNF-α or IL-1β. To determine whether IL-6 is related to CMV-induced intestinal epithelial barrier disruption, an anti-IL-6 neutralizing antibody was used to block IL-6 function. The results of these studies indicate that CMV-associated violation of the integrity of the epithelium of the

colon may be associated to some extent with induced CMV-IL-6. In addition, TNF-α transcripts are expressed in the intestinal mucosa from untreated AIDS patients with CMV colitis and may be associated with macrophage-like cells containing cytomegalovirus inclusions [49]. It is important to note that elevated levels of these proinflammatory cytokines have been associated with CMV IE gene expression, and these cytokines increase the par acellular permeability of epithelial and endothelial cells [26, 50-59]. Interestingly, despite the induction of proinflammatory cytokines early after infection, CMV inhibits TNF-α and IL-1β signaling pathways at a later date, demonstrating unique adaptive capabilities that allow the virus to be stored in the host [60].

In general, these published observations suggest that CMV-induced disruption of intestinal epithelial compounds may be mediated by these proinflammatory cytokine pathways. It is noteworthy that HIV infection does not cause IL-6 expression in vitro [61], although increased plasma IL-6 levels are associated with a risk of HIV disease progression [1, 62-66]. The exact cause of elevated plasma IL-6 in chronic HIV disease remains unclear, but it may be the result of a combination of several factors, one of which may be the constant replication of CMV in different areas of infected individuals. It is equally interesting to consider the role of CMV-induced IL-6 in increased epithelial proliferation in the treatment of ART and in untreated individuals with intestinal epithelial barrier dysfunction [3]. As previously reported, disruption of polarized endothelial cell junctions by CMV causes proliferation of other endothelial cells [25], indicating a balance between lysis of infected endothelial cells and replacement of uninfected ones [67]. It has also been reported that the CM28 chemo toxin receptor encoded by CMV mediates cell proliferation through activation of the IL-6-STAT3 signaling axis [68] and that CMV secretion promotes angiogenesis and lymph angiogenesis through activation of IL-6 production [69, 70]. Based on these published data, we hypothesize that CMV infection of the intestinal epithelium may cause IL-6 production, resulting in increased cell proliferation to replace infected intestinal cells. These findings suggest that CMV does not lyse all cells of polarized intestinal cells. The infected cells can be replaced by non-polar proliferating cells, creating small foci of CMV infection with compromised narrow joints that can locally facilitate bacterial translocation, leading to persistent inflammation [4]. AIDS-associated gastrointestinal disease CMV generally responds well to ganciclovir and foscarnet [71, 72-75]. However, the effect of CMV on intestinal epithelial connections in the presence of currently approved or new drugs has never been studied before, which creates new prospects for studying this problem as a basis for developing new treatments for HIV/CMV coinfection.

References

1. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008; 5(10):e203.
2. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, et al. 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. 2009; 199(8):1177±85.
3. Somsouk M, Estes JD, Deleage C, Dunham RM, Albright R, Inadomi JM, et al. Gut epithelial barrier and systemic inflammation during chronic HIV infection. Aids. 2015; 29(1):43±51
4. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Science translational medicine. 2013; 5(193):193ra91
5. Lichtner M, Cicconi P, Vita S, Cozzi-Lepri A, Galli M, Lo Caputo S, et al. CMV co-infection is associated with increased risk of Severe non-AIDS events in a large cohort of HIV-infected patients. J Infect Dis 2014.
6. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease

occurs predominantly in the gastrointestinal tract. J Exp Med. 2004; 200(6):749±59.
7. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNeil A, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol. 2003; 77(21):11708±17.
8. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med. 2004; 200(6):761±70.
9. Mehandru S, Poles MA, Tenner-Racz K, Jean-Pierre P, Manuelli V, Lopez P, et al. Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med.2006; 3(12):e484.
10. Emery VC. Investigation of CMV disease in immunocompromised patients. J Clin Pathol. 2001; 54 (2):84±8.
11. Steininger C, Puchhammer-Stockl E, Popow-Kraupp T. Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART). Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology. 2006; 37(1):1±9.
12. Hunt PW, Martin JN, Sinclair E, Epling L, Teague J, Jacobson MA, et al. Valganciclovir reduces T cell activation in HIV-infected individuals with incomplete CD4+ T cell recovery on antiretroviral therapy. J Infect Dis. 2011; 203(10):1474±83.
13. Sylwester AW, Mitchell BL, Edgar JB, Taormina C, Pelte C, Ruchti F, et al. Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. J Exp Med. 2005; 202(5):673±85.
14. Freeman ML, Lederman MM, Gianella S. Partners in Crime: The Role of CMV in Immune Dysregulation and Clinical Outcome During HIV Infection. Curr HIV/AIDS Rep. 2016; 13(1):10±9.
15. Pawelec G. Immunosenenescence: role of cytomegalovirus. Experimental gerontology. 2014; 54:1±5.
16. Sansoni P, Vescovini R, Fagnoni FF, Akbar A, Arens R, Chiu YL, et al. New advances in CMV and immunosenescence. Experimental gerontology. 2014; 55:54±62.
17. Hsue PY, Hunt PW, Sinclair E, Bredt B, Franklin A, Killian M, et al. Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. Aids. 2006; 20(18):2275±83.
18. Lichtner M, Cicconi P, Vita S, Cozzi-Lepri A, Galli M, Lo Caputo S, et al. Cytomegalovirus coinfection is associated with an increased risk of severe non-AIDS-defining events in a large cohort of HIVinfected patients. J Infect Dis. 2015; 211(2):178±86.
19. Sacre K, Hunt PW, Hsue PY, Maidji E, Martin JN, Deeks SG, et al. A role for cytomegalovirus-specific CD4+CX3CR1+ T cells and cytomegalovirus-induced T-cell immunopathology in HIV-associated atherosclerosis. Aids. 2012; 26(7):805±14.
20. Kempen JH, Jabs DA, Wilson LA, Dunn JP, West SK, Tonascia J. Mortality risk for patients with cytomegalovirus retinitis and acquired immune deficiency syndrome. Clin Infect Dis. 2003; 37(10):1365±73.
21. Deayton JR, Prof Sabin CA, Johnson MA, Emery VC, Wilson P, Griffiths PD. Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet. 2004; 363(9427):2116±21.
22. Francis ND, Boylston AW, Roberts AH, Parkin JM, Pinching AJ. Cytomegalovirus infection in gastrointestinal tracts of patients infected with HIV-1 or AIDS. J Clin Pathol. 1989; 42(10):1055±64.
23. Culpepper-Morgan JA, Kotler DP, Scholes JV, Tierney AR. Evaluation of diagnostic criteria for mucosal cytomegalic inclusion disease in the acquired immune deficiency syndrome. The American journal of gastroenterology. 1987; 82(12):1264±70.

24. Meiselman MS, Cello JP, Margaretten W. Cytomegalovirus colitis. Report of the clinical, endoscopic, and pathologic findings in two patients with the acquired immune deficiency syndrome. Gastroenterology. 1985; 88(1 Pt 1):171±5.
25. Maidji E, Percivalle E, Gerna G, Fisher S, Pereira L. Transmission of human cytomegalovirus from infected uterine microvascular endothelial cells to differentiating/invasive placental cytotrophoblasts. Virology. 2002; 304(1):53±69
26. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J Biol Chem. 2011; 286(36):31263±71
27. Geist LJ, Monick MM, Stinski MF, Hunninghake GW. The immediate early genes of human cytomegalovirus upregulate tumor necrosis factor-alpha gene expression. J Clin Invest. 1994; 93(2):474±8.
28. Crump JW, Geist LJ, Auron PE, Webb AC, Stinski MF, Hunninghake GW. The immediate early genes of human cytomegalovirus require only proximal promoter elements to upregulate expression of interleukin-1 beta. American journal of respiratory cell and molecular biology. 1992; 6(6):674±7.
29. Geist LJ, Dai LY. Cytomegalovirus modulates interleukin-6 gene expression. Transplantation. 1996;62(5):653±8.
30. Carlquist JF, Edelman L, Bennion DW, Anderson JL. Cytomegalovirus induction of interleukin-6 in lung fibroblasts occurs independently of active infection and involves a G protein and the transcription factor, NF-kappaB. J Infect Dis. 1999; 179(5):1094±100.
31. Almeida GD, Porada CD, St Jeor S, Ascensao JL. Human cytomegalovirus alters interleukin-6 production by endothelial cells. Blood. 1994; 83(2):370±6.
32. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, et al. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. Journal of immunology. 2003; 171(11):6164±72.
33. Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. Biochimica et biophysica acta. 2009;1788(4):864±71.
34. Naydenov NG, Baranwal S, Khan S, Feygin A, Gupta P, Ivanov AI. Novel mechanism of cytokineinduced disruption of epithelial barriers: Janus kinase and protein kinase D-dependent downregulation of junction protein expression. Tissue barriers. 2013; 1(4):e25231.
35. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006; 12(12):1365±71.
36. Marchetti G, Bellistri GM, Borghi E, Tincati C, Ferramosca S, La Francesca M, et al. Microbial translocation is associated with sustained failure in CD4+ T-cell reconstitution in HIV-infected patients on long-term highly active antiretroviral therapy. Aids. 2008; 22(15):2035±8.
37. Cassol E, Malfeld S, Mahasha P, van der Merwe S, Cassol S, Seebregts C, et al. Persistent microbial translocation and immune activation in HIV-1-infected South Africans receiving combination antiretroviral therapy. J Infect Dis. 2010; 202(5):723±33.
38. Griffiths PD. CMV as a cofactor enhancing progression of AIDS. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology. 2006; 35(4):489±92.
39. Gianella S, Anderson CM, Var SR, Oliveira MF, Lada SM, Vargas MV, et al. Replication of Human Herpesviruses Is Associated with Higher HIV DNA Levels during Antiretroviral Therapy Started at Early Phases of HIV Infection. J Virol. 2016; 90(8):3944±52.
40. Gianella S, Massanella M, Richman DD, Little SJ, Spina CA, Vargas MV, et al. Cytomegalovirus replication in semen is associated with higher levels of proviral HIV DNA and CD4+ T cell activation during antiretroviral treatment. J Virol. 2014; 88(14):7818±27.

41. Adland E, Klenerman P, Goulder P, Matthews PC. Ongoing burden of disease and mortality from HIV/CMV coinfection in Africa in the antiretroviral therapy era. Front Microbiol. 2015; 6:1016.
42. Caby F, Guihot A, Lambert-Niclot S, Guiguet M, Boutolleau D, Agher R, et al. Determinants of a Low CD4/CD8 Ratio in HIV-1-Infected Individuals Despite Long-term Viral Suppression. Clin Infect Dis. 2016; 62(10):1297±303.
43. Dupont L, Reeves MB. Cytomegalovirus latency and reactivation: recent insights into an age old problem. Rev Med Virol. 2016; 26(2):75±89
44. Golan L, Livneh-Kol A, Gonen E, Yagel S, Rosenshine I, Shpigel NY. Mycobacterium avium paratuberculosis invades human small-intestinal goblet cells and elicits inflammation. J Infect Dis. 2009; 199(3):350±4.
45. Zhang Z, Jin L, Champion G, Seydel KB, Stanley SL Jr. Shigella infection in a SCID mouse-human intestinal xenograft model: role for neutrophils in containing bacterial dissemination in human intestine. Infection and immunity. 2001; 69(5):3240±7.
46. Gibbons C, Kollmann TR, Pettoello-Mantovani M, Kim A, Goldstein H. Thy/Liv-SCID-Hu mice implanted with human intestine: an in vivo model for investigation of mucosal transmission of HIV. AIDS research and human retroviruses. 1997; 13(17):1453±60.
47. Savidge TC, Morey AL, Ferguson DJ, Fleming KA, Shmakov AN, Phillips AD. Human intestinal development in a severe-combined immunodeficient xenograft model. Differentiation; research in biological diversity. 1995; 58(5):361±71.
48. Lin WR, Su MY, Hsu CM, Ho YP, Ngan KW, Chiu CT, et al. Clinical and endoscopic features for alimentary tract cytomegalovirus disease: report of 20 cases with gastrointestinal cytomegalovirus disease. Chang Gung Med J. 2005; 28(7):476±84.
49. Smith PD, Saini SS, Raffeld M, Manischewitz JF, Wahl SM. Cytomegalovirus induction of tumor necrosis factor-alpha by human monocytes and mucosal macrophages. J Clin Invest. 1992; 90(5):1642±8.
50. Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA, et al. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. American journal of physiology Gastrointestinal and liver physiology. 2004; 286(3):G367±76.
51. Ye D, Ma I, Ma TY. Molecular mechanism of tumor necrosis factor-alpha modulation of intestinal epithelial tight junction barrier. American journal of physiology Gastrointestinal and liver physiology. 2006; 290(3):G496±504.
52. Ozaki H, Ishii K, Horiuchi H, Arai H, Kawamoto T, Okawa K, et al. Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. Journal of immunology. 1999; 163(2):553±7.
53. Blum MS, Toninelli E, Anderson JM, Balda MS, Zhou J, O'Donnell L, et al. Cytoskeletal rearrangement mediates human microvascular endothelial tight junction modulation by cytokines. Am J Physiol. 1997; 273(1 Pt 2):H286±94.
54. Al-Sadi R, Guo S, Ye D, Ma TY. TNF-alpha modulation of intestinal epithelial tight junction barrier is regulated by ERK1/2 activation of Elk-1. Am J Pathol. 2013; 183(6):1871±84.
55. Al-Sadi R, Guo S, Ye D, Dokladny K, Alhmoud T, Ereifej L, et al. Mechanism of IL-1beta modulation of intestinal epithelial barrier involves p38 kinase and activating transcription factor-2 activation. Journal of immunology. 2013; 190(12):6596±606.
56. Al-Sadi R, Ye D, Boivin M, Guo S, Hashimi M, Ereifej L, et al. Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. PLoS One. 2014; 9(3):e85345.

57. Al-Sadi RM, Ma TY. IL-1beta causes an increase in intestinal epithelial tight junction permeability.Journal of immunology. 2007; 178(7):4641±9.
58. Al-Sadi R, Ye D, Said HM, Ma TY. IL-1beta-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-kappaB pathway. Am J Pathol. 2010; 177 (5):2310±22.
59. Shirasawa M, Sonoda S, Terasaki H, Arimura N, Otsuka H, Yamashita T, et al. TNF-alpha disrupts morphologic and functional barrier properties of polarized retinal pigment epithelium. Exp Eye Res. 2013; 110:59±69.
60. Jarvis MA, Borton JA, Keech AM, Wong J, Britt WJ, Magun BE, et al. Human cytomegalovirus attenuates interleukin-1beta and tumor necrosis factor alpha proinflammatory signaling by inhibition of NFkappaB activation. J Virol. 2006; 80(11):5588±98.
61. Shive CL, Biancotto A, Funderburg NT, Pilch-Cooper HA, Valdez H, Margolis L, et al. HIV-1 is not a major driver of increased plasma IL-6 levels in chronic HIV-1 disease. Journal of acquired immune deficiency syndromes. 2012; 61(2):145±52.
62. Kalayjian RC, Machekano RN, Rizk N, Robbins GK, Gandhi RT, Rodriguez BA, et al. Pretreatment levels of soluble cellular receptors and interleukin-6 are associated with HIV disease progression in subjects treated with highly active antiretroviral therapy. J Infect Dis. 2010; 201(12):1796±805.
63. Breen EC, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, et al. Infection with HIV is associated with elevated IL-6 levels and production. Journal of immunology. 1990; 144(2):480±4.
64. Lafeuillade A, Poizot-Martin I, Quilichini R, Gastaut JA, Kaplanski S, Farnarier C, et al. Increased interleukin-6 production is associated with disease progression in HIV infection. Aids. 1991; 5(9):1139±40.
65. Hsu DC, Ma YF, Hur S, Li D, Rupert A, Scherzer R, et al. Plasma IL-6 levels are independently associated with atherosclerosis and mortality in HIV-infected individuals on suppressive ART. Aids. 2016.
66. French MA, Cozzi-Lepri A, Arduino RC, Johnson M, Achhra AC, Landay A, et al. Plasma levels of cytokines and chemokines and the risk of mortality in HIV-infected individuals: a case-control analysis nested in a large clinical trial. Aids. 2015; 29(7):847±51.
67. Kahl M, Siegel-Axel D, Stenglein S, Jahn G, Sinzger C. Efficient lytic infection of human arterial endothelial cells by human cytomegalovirus strains. J Virol. 2000; 74(16):7628±35.
68. Slinger E, Maussang D, Schreiber A, Siderius M, Rahbar A, Fraile-Ramos A, et al. HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. Sci Signal. 2010; 3(133):ra58.
69. Botto S, Streblow DN, DeFilippis V, White L, Kreklywich CN, Smith PP, et al. IL-6 in human cytomegalovirus secretome promotes angiogenesis and survival of endothelial cells through the stimulation of survivin. Blood. 2011; 117(1):352±61.
70. Fiorentini S, Luganini A, Dell'Oste V, Lorusso B, Cervi E, Caccuri F, et al. Human cytomegalovirus productively infects lymphatic endothelial cells and induces a secretome that promotes angiogenesis and lymphangiogenesis through interleukin-6 and granulocyte-macrophage colony-stimulating factor. J Gen Virol. 2011; 92(Pt 3):650±60.
71. Blanshard C, Benhamou Y, Dohin E, Lernestedt JO, Gazzard BG, Katlama C. Treatment of AIDSassociated gastrointestinal cytomegalovirus infection with foscarnet and ganciclovir: a randomized comparison. J Infect Dis. 1995; 172(3):622±8.
72. Parente F, Bianchi Porro G. Treatment of cytomegalovirus esophagitis in patients with acquired immune deficiency syndrome: a randomized controlled study of foscarnet versus ganciclovir. The Italian Cytomegalovirus Study Group. The American journal of gastroenterology. 1998; 93(3):317±22.

73. Maidji E. et al. Replication of CMV in the gut of HIV-infected individuals and epithelial barrier dysfunction //PLoS pathogens. – 2017. – Т. 13. – №. 2. – С. e1006202.
74. Hirao L. A. et al. Early mucosal sensing of SIV infection by paneth cells induces IL-1β production and initiates gut epithelial disruption //PLoS pathogens. – 2014. – Т. 10. – №. 8. – С. e1004311.
75. Sindberg G. M. Disruption of Gut Homeostasis by Opioids in the Early Stages of HIV Infection : дис. – University of Minnesota, 2014.