Experimental and Molecular Pathology 97 (2014) 225–233

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Associated changes in the transcription levels of IL-17A and tight junction-associated genes in the duodenal mucosa of rhesus macaques repeatedly exposed to simian/human immunodeficiency virus

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ARTICLE INFO

Article history:
Received 8 July 2014
Available online 14 July 2014

Keywords:
Tight junction
Claudins
Occludin
Zonula occluden-1
IL-17A
SHIV
Chinese rhesus macaques

ABSTRACT

Background: Mucosal barrier dysfunction might play a key role in HIV/AIDS, yet the early effects of HIV-1 on intestinal mucosal barrier, especially tight junctions (TJ) have not been well addressed.
Aims: To investigate the effects of acute HIV-1 infection on the expression of intestinal IL-17A and TJ-associated genes using an NHP-AIDS model.
Methods: TaqMan probe real-time RT-PCR methods were established and claudin-1, claudin-3, occludin and zonula occluden-1 (ZO-1) mRNA levels in the duodenal biopsies of rhesus macaques collected before and after rectal exposures to SHIV-SF162P4 were examined and compared with that of IL-17A, IL-6, TGF-β, RORγt, T-bet, Foxp-3 and GATA-3.
Results: The mRNA levels of TJ-associated genes were statistically significantly reduced soon after viral exposures and the mRNA levels of claudin-1, occludin and ZO-1 in viral positive tissues (from Group I) were lower than that in viral negative tissues (from Group II) after viral exposure. IL-17A mRNA levels were also decreased and positively correlated with the mRNA levels of the TJ-associated genes after viral exposure or infection, although the levels of IL-6, TGF-β and RORγt mRNA showed no statistical difference. The levels of GATA-3 mRNA in tissues collected before viral exposure were statistically different between Group I and Group II animals. The balance between T-bet and GATA-3 mRNA levels in Group II was markedly altered and statistically significantly different from that in Group I.
Conclusions: Acute SHIV, and by extension HIV infection could affect the expression of TJ-associated genes, probably through IL-17A and other immune alterations.

Introduction

Microbial translocation and chronic activation of the immune system are major driving forces of HIV/AIDS disease progression (Marchetti et al., 2013). It was found that the magnitude of HIV/SIV-associated chronic immune activation could predict disease progression much better than either CD4 + T cell count or plasma viral load (Estes et al., 2010; Giorgi et al., 1999; Sodora and Silvestri, 2008). In line with this, low levels of immune activation were observed in chronically infected natural hosts of SIV, such as African green monkeys and Sooty mangabeys who do not progress to diseases despite high levels of virus replication (Pandrea et al., 2008; Silvestri et al., 2003). Microbial translocation from the intestinal lumen to the systemic circulation appears to be a key factor underlying the chronic immune activation, and it is supposed to be the consequence of intestinal mucosal barrier dysfunction (Brenchley et al., 2006; Klatt et al., 2012). However, the underlying mechanisms such as the causal relationships between virus infection, intestinal mucosal barrier dysfunction and translocation of microbes and microbial products, and when and how the earliest gut barrier dysfunction occurred have not been completely understood. Such information may facilitate the development of novel adjunctive treatment strategies to decrease microbial translocation and subsequent immune activation, therefore decrease the morbidity and mortality in HIV infection.

It has been shown that acute HIV-1 infection could affect intestinal barrier function through mediating epithelial cell apoptosis (Epple et al., 2010; Sankaran et al., 2008); however, it remains unknown whether the acute effects on intestinal barrier could also be mediated through alteration of the tight junctions. Tight junctions (TJ) between epithelial cells of the intestine are key element of the physical intestinal barrier. This structure not only ensures cell polarity by inhibiting diffusion of membrane proteins between the apical and the basolateral

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http://dx.doi.org/10.1016/j.yexmp.2014.07.007
0014-4800/

side, but also functions as a gateway determining the permeability of the epithelial cell layer via the paracellular pathway. TJs are consisted of multiple specialized proteins, including claudins, occludin and zona occludens. Alterations in the expression of TJ-associated genes have been associated with a variety of diseases such as inflammatory bowel diseases and bacterial and viral infections (Epple et al., 2009; Ohnemus et al., 2008; Zeissig et al., 2007). Intestinal barrier dysfunctions have been indicated long since the early studies on AIDS (Sharpstone et al., 1999). TJ abnormality has also been observed in chronic HIV-1 infection of humans and SIV infection of Asian macaques (Milush et al., 2011; Chaudhuri et al., 2008; Maclean et al., 2005). However, studies on the roles of tight junction defects in the early phase of infection were rare and such studies could facilitate the understanding and treatment of gut barrier dysfunction in HIV/AIDS.

IL-17A is the signature cytokine of Th17 cells, the differentiation of which is controlled by nuclear transcription factor RORγt and determined by the availability of cytokines such as TGF-β and IL-6 in humans (Manel et al., 2008; Yang et al., 2008; Zhou et al., 2008). As has been well established that CD4 + T cells, including Th1, Th2, Tregs and Th17, were dramatically depleted soon after HIV-1 infection; and Th17 cells were preferentially depleted in the intestinal mucosa (Brenchley et al., 2008; Veazey et al., 1998). It has also been shown that IL-17A could affect the expression of TJ-associated genes (Kinugasa et al., 2000). Therefore, it is possible that IL-17A have played a role in the dysfunction of intestinal barrier integrity in HIV/AIDS (Dandekar et al., 2010). However, little is known about the relationship between IL-17A and TJ-associated genes in the intestinal mucosa, especially in the context of acute HIV-1 infection.

Simian/human immunodeficiency viruses (SHIV) have been instrumental in a variety of studies, such as HIV-1 gene function, AIDS pathogenesis, and AIDS vaccine researches (Joag et al., 1996; Li et al., 1995; Reimann et al., 1996). SHIV-SF162P4 was constructed with SIVmac239 and HIV-1 SF162 (Hsu et al., 2003; Luciw et al., 1995), and it has been widely used to test candidate AIDS vaccines, especially those intended for stopping mucosal transmission of viruses (Bomsel et al., 2011; Yang et al., 2012). In vitro experiments have shown that culture supernatant of HIV-1 SF162 could alter TJs and gp120 of HIV-1 could directly affect TJ expression (Schmitz et al., 2002), which indicated that SHIV-SF162P4 infection of rhesus macaques might be a useful model for studying the mechanism of intestinal barrier dysfunction in HIV/AIDS. To find out whether acute HIV-1 infection could affect the expression of TJ-associated genes in vivo and if so, the possible link to IL-17A expression in the same tissues, SHIV/rhesus model was used and duodenal biopsies from the rhesus macaques before and after exposure to SHIV-SF162P4 were examined for the mRNA levels of TJ-associated genes, i.e., claudin-1, claudin-3, occludin and zona occluden (ZO-1). The relations between the mRNA levels of TJ-associated genes and that of IL-17A and genes associated with its expression were also examined.

Materials and methods

Duodenal mucosal tissues

Duodenal biopsies were obtained as described previously (Zhang et al., 2012). Briefly rhesus macaques (*Macaca mulatta*) were anesthetized and 5 pinches of duodenal tissues from each animal were obtained with pediatric gastrointestinal endoscopes and biopsy forceps (Olympus, Japan). All the tissues were frozen on dry ice immediately after collection, transferred to the laboratory and preserved in a −70 °C freezer before use. Sample collections were performed at 2 weeks before and 10 days after the animals were repeatedly exposed to SHIV-SF162P4 via rectal route. The animals were free of SIV, STLV and SRV before the experiments. All the animals were of Chinese origin and handled humanely according to our state and local laws and regulations on the care and use of laboratory animals.

RNA isolation

Total RNA was isolated with RNeasy mini kit (Qiagen, Germany) or using RNAprep Tissue kits (Tiangen Biotech, Beijing) according to manufacturers' protocols. Briefly (for the latter kit), the tissues were homogenized in lysing buffer and digested with proteinase K. The supernatants were mixed with ethanol and loaded into CR3 column. After washing with RW1 and digestion with RNase-Free DNase I, the columns were washed with RW1 again and then with RW for 2 times. RNA was washed into RNase-Free ddH_2O, divided into small aliquots and freezed at −70 °C for future use.

RNA standards preparation

RNA standards for quantitative RT-PCR were prepared as described previously (Yu et al., 2010). First, nucleotide sequence fragments for tight junction associated-genes including claudin-1, claudin-3, occludin, and zona occluden-1, for cytokines including IL-17A, IL-6 and TGF-β, and for nuclear transcription factors including RORγt, T-bet, GATA-3, and Foxp-3 were amplified by RT-PCR with TaKaRa One Step RNA PCR Kit (AMV) (Dalian, China) and sub-cloned into pGEM®-T Easy vector (Promega, USA). Plasmids containing the correct nucleotide sequences for these genes were propagated in Top 10, purified, linearized and the RNA for each of them was obtained by in vitro transcription using RiboMAX™ Large Scale RNA Production System (Promega, USA). Obtained RNA standards were quantified by spectrometry and 10× serial dilutions were made and preserved in aliquots at −70 °C.

Quantification of TJ gene mRNAs

TaqMan probe real-time RT-PCR systems for detection of claudin-1, claudin-3, occludin, ZO-1 and other genes were set up with One Step PrimeScript® RT-PCR Kit (Takara, Dalian, China) as described previously (Lei et al., 2013). The primers and probes used were listed in Table 1. Briefly, reaction mixtures were made using reagents included in the kit and probes and primers synthesized by a service provider (Invitrogen or Sangon Biotech, Shanghai, China). Reactions were carried out with Thermal Cycler Dice® Real Time System TP800 with Software version 1.03B (Takara Bio Inc., Japan).

Table 1
Primers and probes for the amplification of tight junction associated genes.

Primer or probe name	Sequence 5′–3′	Reference sequence
Claudin-1 primer F	GCGACAACATCGTGAC	NM_001193969.1
Claudin-1 primer R	ACAGGAACAGCAAAGTAG	
q claudin-1 primer F	GTCTTTGACTCCTTGCTGAATCTG	
q claudin-1 primer R	TTGCTATCACTCCCAGGAGGAT	
q claudin-1 probe	CAGCACATTGCAAGCAACCCGTG	
Claudin-3 primer F	GGTCCGCCAACACCATTATCCG	NM_001194562.1
Claudin-3 primer R	TCCTTGCGGTCGTAGGCTGTG	
q claudin-3 primer F	GTCCGCCAACACCATTATCC	
q claudin-3 primer R	GTACAGGCTCGTGCCCATCT	
q claudin-3 probe	TACAACCCCGTGGTGCCTGAGGC	
Occludin primer F	GCCGTTACTGGACTCTAC	XM_002804403.1
Occludin primer R	AGGCGAAGTCACTGGAAGC	
q occludin primer F	GCGGAAAGAGTTGACAGT	
q occludin primer R	CCGGTGTGGATTTATAGG	
q occludin probe	TGGCATACTCTTCCAACGGC	
ZO-1 primer F	CGGATGGTGCTACAAGTG	XM_002804692.1
ZO-1 primer R	CAGGGGAGTCTATTCTATG	
q ZO-1 primer F	ACAAGTGATGACCTTGATTTGCAT	
q ZO-1 primer R	GTCTACTGTCCGTGCTATACATTGAGT	
q ZO-1 probe	ATGATCGTCTGTCCTACCTGTCAGCTCCA	

Note: Names that begin with a "q" are names of primers and probes for quantitative (real-time) RT-PCR.