Expression of plgR in the tracheal mucosa of SHIV/SIV-infected rhesus macaques

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ABSTRACT

Polymeric immunoglobulin receptors (plgR) are key participants in the formation and secretion of secretory IgA (S-IgA), which is critical for the prevention of microbial infection and colonization in the respiratory system. Although increased respiratory colonization and infections are common in HIV/AIDS, little is known about the expression of plgR in the airway mucosa of these patients. To address this, the expression levels of plgR in the tracheal mucosa and lungs of SHIV/SIV-infected rhesus macaques were examined by real-time RT-PCR and confocal microscopy. We found that the levels of both PIGR mRNA and plgR immunoreactivity were lower in the tracheal mucosa of SHIV/SIV-infected rhesus macaques than that in non-infected rhesus macaques, and the difference in plgR immunoreactivity was statistically significant. IL-17A, which enhances plgR expression, was also changed in the same direction as that of plgR. In contrast to changes in the tracheal mucosa, plgR and IL-17A levels were higher in the lungs of infected rhesus macaques. These results indicated abnormal plgR expression in SHIV/SIV, and by extension HIV infections, which might partially result from IL-17A alterations and might contribute to the increased microbial colonization and infection related to pulmonary complications in HIV/AIDS.

Keywords: Tracheal mucosa; Lungs; plgR; SHIV/SIV infection; IL-17A

INTRODUCTION

The respiratory system is continuously exposed to foreign antigens from either airborne or commensal microbes. Due to vulnerability of the physical epithelial barrier of the respiratory system, most pathogens are stopped from entering the body by the mucosal immune system. A key component of the airway mucosal immune system that prevents microbial infections and colonization is secretory IgA (S-IgA), which is composed of dimeric IgA produced in the lamina propria and extracellular part of the polymeric immunoglobulin receptors (plgR), also known as the secretory component (SC) expressed by mucosal epithelial cells (Johansen & Kaetzel, 2011).

Newly synthesized plgR is localized to the basolateral surfaces of mucosal epithelial cells, where it binds to dimeric IgA (dlgA) and mediates transcytosis of IgA to the apical surface of the epithelial cells (Johansen et al., 1999). The SC can be released to the mucosal surface alone (in the absence of IgA) or together with dlgA as S-IgA. In addition, SC bound to dlgA can elongate the life of S-IgA and enhance its immune exclusion ability. It can also stop microbial invasion. Mice deficient in plgR expression are reportedly unable to control infections of the airway by some bacteria, which could drive progressive chronic obstructive pulmonary disease (COPD) phenotype in these mice (Richmond et al., 2016).

Pulmonary complications are common and major causes of morbidity and mortality in HIV-infected individuals, even in the presence of highly active antiretroviral therapy (ART) (Grubb et al., 2006; Murray, 1996). Increased pulmonary infections and microbial colonization are common in HIV/AIDS patients (Zar, 2008). Whether and how the S-IgA/plgR system is involved in these alterations is not well addressed. Rhesus macaques are important in HIV/AIDS studies. In previous research, we found that plgR expression was altered in the gut mucosa of SHIV/SIV-infected rhesus macaques (Wang & Yang, 2016). To determine whether plgR is involved in the respiratory pathology of HIV/AIDS, we examined the expression of plgR in the tracheal mucosa of SHIV/SIV-infected rhesus macaques.

MATERIALS AND METHODS

Tissues

Tissue samples from the tracheas and lungs were collected from five normal and five SHIV/SIV-infected rhesus macaques.
All quantitative parameters were expressed as mean±SD. Non-parametric Mann-Whitney U test was used to compare the means of parameters between normal and infected rhesus macaques. Spearman test was used to calculate the correlations between plgR mRNA and IL-17A mRNA levels. P values of less than 0.05 were considered statistically significant.

RESULTS

Localization of plgR immunoreactivity in the tracheal mucosa of rhesus macaques
To detect the expression of plgR in the tracheal mucosa of rhesus macaques, plgR immunoreactive cells were examined by confocal microscopy. As shown in Figure 1, plgR immunoreactivity was detected with a polyclonal antibody against human plgR. In the epithelium, immunoreactivity to plgR was localized to both the apical and basolateral surfaces of the epithelial cells. It was also localized in the cytoplasm of the basal part (under the nucleus) of the epithelial cells. After SHIV/SIV infection, plgR immunoreactivity was lower in the tracheal mucosa of rhesus macaques.

Expression of plgR decreased in the tracheal epithelium of SHIV/SIV-infected rhesus macaques
To determine changes in plgR expression after SHIV/SIV infection, levels of plgR immunoreactivity were quantitatively examined with Image-Pro Plus software and plgR mRNA levels were examined by real-time PCR. As shown in Figure 2, levels of plgR immunoreactivity were 1.65 times higher in the tracheal epithelium of normal rhesus macaques than that in SHIV/SIV-infected rhesus macaques (Figure 2A), with statistical significance (Mann-Whitney U test, P=0.007 9). The transcription levels of plgR genes in the tracheal mucosa of normal rhesus macaques were 1.57 times higher than that in infected rhesus macaques, although the difference was not statistically significant (Mann-Whitney U test, P=0.254 4). Therefore, both the transcription and protein levels of plgR were about 1.6 times higher in normal than in infected rhesus macaques.

IL-17A is a regulator of plgR expression and is decreased in HIV and SIV infection. We examined the transcription levels of IL-17A in the tracheal mucosa of normal and infected rhesus macaques. IL-17A mRNA levels in the tracheal mucosa of normal rhesus macaques were 1.8 times higher than that in SHIV/SIV-infected rhesus macaques (Figure 2C), although the difference did not reach statistical significance (Mann-Whitney U test, P=0.5476). Positive correlation was observed between plgR and IL-17A mRNA levels in the tracheal mucosa of normal rhesus macaques (Figure 2D), though this trend was not found in SHIV/SIV-infected rhesus macaques.

Expression of plgR in the lungs of SHIV/SIV-infected rhesus macaques
To determine whether the lungs of SHIV/SIV-infected rhesus macaques were similarly affected, the expressions of plgR mRNA and IL-17A mRNA in the lungs of normal and infected rhesus macaques were examined. The mRNA levels of plgR and IL-17A were 50 and 32 times higher, respectively, in the tracheal mucosa than in the lungs. As shown in Figure 3, plgR and IL-17A mRNA were both detected in the lungs of normal and infected rhesus macaques. In contrast to the changes observed in the tracheal mucosa, the levels of plgR and IL-17A mRNA were 3 and 1.2 times higher, respectively, in infected rhesus macaques than in normal rhesus macaques, although the differences were not statistically significant (Mann-Whitney U test, P=0.4396 and 0.7857, respectively). Therefore, the expressions of plgR and IL-17A were higher in the tracheal mucosa than in the lungs, and were not reduced in the lungs of SHIV/SIV-infected rhesus macaques.

DISCUSSION

In the present study, we observed reduced expression of plgR
Figure 1 Distribution of plgR immunoreactivity in the epithelium of tracheal mucosa from rhesus macaques

Immunoreactivities to plgR (green) in the pseudo-stratified columnar epithelium of tracheal mucosa from normal (left column) and SHIV/SIV-infected (right column) rhesus macaques are shown. Nuclei (blue, stained with DAPI) and DIC images indicate that histological alterations also occurred in the tracheal mucosa of SHIV/SIV-infected rhesus macaques. Original magnification, ×800.

in the tracheal mucosa of SHIV/SIV-infected rhesus macaques. Both the protein levels and mRNA levels of plgR were decreased to almost the same degree, although the decrease in protein levels was statistically significant, whereas that of mRNA was not. It is possible that the effects of SHIV/SIV infection on plgR expression were at the gene transcription level. In consistent with these results, previous research showed that plgR mRNA levels were significantly reduced in the intestinal mucosa of SHIV/SIV-infected rhesus macaques (Wang & Yang, 2016). Downregulation of plgR in airway mucosa has also been documented in other airway diseases (Gohy et al., 2014; Hupin et al., 2013). Since the pathology between SIV and HIV infection is similar, plgR expression in the airway mucosa of HIV-infected patients could also be significantly affected.

The mechanism of decreased plgR expression in SHIV/SIV infection has not been addressed. There are many potential factors that could affect plgR expression, among which IL-17A can significantly regulate plgR expression (Jaffar et al., 2009). In the present study, a decrease in IL-17A expression in the tracheal mucosa of infected rhesus macaques was observed, suggesting a role of IL-17A in the downregulation of plgR expression in the context of SHIV/SIV infection. The non-significant difference might be due to the large individual variability and small sample size. Significant correlation between plgR and IL-17A mRNA has been observed in the intestinal mucosa of these animals and a significant decrease in IL-17A mRNA has also been observed in the intestinal mucosa (Wang & Yang, 2016; Zhang et al., 2014). Further studies are warranted to reveal the mechanism underlying the decrease of plgR expression in HIV/AIDS.
The consequence of reduced plgR expression in the tracheal mucosa of SHIV/SIV-infected rhesus macaques is unknown. Nevertheless, these data indicate impaired immune exclusion of potential pathogenic and commensal microbes in the respiratory system. In line with this, increased airway microbes and pulmonary infections have been documented in HIV/SIV infections (Nimmo et al., 2015; Twigg et al., 2016). Since elevated microbes can drive the COPD-like phenotype in plgR
deficient mice (Richmond et al., 2016) and downregulation of plgR is observed in COPD patients (Gohy et al., 2014), reduced plgR expression could be an underlying mechanism of the increased incidence of COPD in HIV/AIDS patients (Morris et al., 2011). COPD is the cause of death in a significant proportion of the HIV/AIDS population. ART treatment does not decrease the incidence of COPD, but is an independent predictor of increased airway obstruction (Gingo et al., 2010). Decreased expression of plgR might also be involved in other pathological processes of HIV/AIDS, such as lung cancer (Ocak et al., 2012). Therefore, abnormal expression of plgR should be taken into consideration in novel therapies for pulmonary complications such as COPD.

In summary, for the first time, reduced plgR expression was observed in the tracheal mucosa of SHIV/SIV-infected rhesus macaques, which might be linked to IL-17A reduction in the tracheal mucosa. The reduced expression of plgR might be the underlying mechanism of increased pulmonary microbiota and infections in HIV/AIDS. Rhesus macaques are a suitable model for future dissection of the mechanisms underlying respiratory complications in HIV/AIDS.

REFERENCES


