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

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ABSTRACT
Polymeric immunoglobulin receptors (pIgR) 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 pIgR in the airway mucosa of these patients. To address this, the expression levels of pIgR 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 pIgR immunoreactivity were lower in the tracheal mucosa of SHIV/SIV-infected rhesus macaques than that in non-infected rhesus macaques, and the difference in pIgR immunoreactivity was statistically significant. IL-17A, which enhances pIgR expression, was also changed in the same direction as that of pIgR. In contrast to changes in the tracheal mucosa, pIgR and IL-17A levels were higher in the lungs of infected rhesus macaques. These results indicated abnormal pIgR 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; pIgR; 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 (pIgR), also known as the secretory component (SC) expressed by mucosal epithelial cells (Johansen & Kaetzel, 2011).

Newly synthesized pIgR 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 pIgR 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/pIgR system is involved in these alterations is not well addressed. Rhesus macaques are important in HIV/AIDS studies. In previous research, we found that pIgR expression was altered in the gut mucosa of SHIV/SIV-infected rhesus macaques (Wang & Yang, 2016). To determine whether pIgR is involved in the respiratory pathology of HIV/AIDS, we examined the expression of pIgR 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.
(Macaca mulatta), as reported previously (Wang & Yang, 2016). The sites from which samples were collected were chosen randomly. Tissue samples for RNA isolation were frozen on dry ice immediately after collection and preserved in a freezer at -80 °C before use. Tissue samples for confocal microscopy were fixed in 4% paraformaldehyde immediately after collection, then washed and protected with 30% sucrose, and finally embedded in OCT and preserved in a freezer at -80 °C. All study animals were treated humanely per the state and local regulations on the care and use of experimental animals.

Real-time RT-PCR
Quantification of plgR and IL-17A mRNA levels was conducted by TaqMan® probe real-time RT-PCR, as performed previously (Wang & Yang, 2016; Zhang et al., 2014). Briefly, RNA was isolated using a RNAprep Pure Tissue Kit (Tiangen Biotech, China) per the manufacturer’s protocols. Real-time PCR mixtures were established with a One Step PrimeScript® RT-PCR Kit (Takara, Japan) and primers and probes for plgR and IL-17A (Wang & Yang, 2016; Zhang et al., 2014). PCR was performed on a 7500 Real-Time PCR System with 7500 System SDS software version 1.4 (ABI, USA). GAPDH mRNA levels in all samples were used as internal controls.

Confocal microscopy
Tissue sections were cut with a cryostat LEICA CM 1850 (Leica Inc., Germany) to a thickness of 20 microns. After removing the OCT with PBS supplemented with 0.1% Triton X-100 and FSG, the slides were washed with PBST and blocked with 10% normal goat serum for 1 h before incubation in polyclonal antibody against human plgR (rabbit anti-PIGR, 4 µg/mL, Abcam, USA) at 4 °C overnight. Sections were incubated in secondary antibody (Alexa Fluor 488 conjugated goat anti-rabbit IgG, 2 µg/mL, Invitrogen, USA) for 1 h after washing off the extra primary antibody. Slides were washed and mounted with anti-fade mounting medium and observed with an Olympus FV1000-DST confocal microscope (Olympus, Japan). Images (1024×1024) were acquired and morphometric measurements were obtained with Image-Pro Plus software version 6.0 (Media Cybernetics, Silver Springs, MD, USA).

Statistics
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 with 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 mRNA and IL-17A mRNA were similar in infected 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...
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 also 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.
Figure 2  Expression levels of pIgR and IL-17A in the tracheal mucosa of rhesus macaques
Levels of pIgR immunoreactivity (A), pIgR mRNA (B) and IL-17A mRNA (C) in the tracheal mucosa of normal and infected rhesus macaques are shown. mRNA levels of IL-17A and pIgR are positively correlated (D).

Figure 3  Expression of pIgR and IL-17A in the lungs of rhesus macaques
mRNA levels of pIgR (A) and IL-17A (B) in the lungs of normal and infected rhesus macaques are shown. Correlation between transcription levels of pIgR and IL-17A in normal rhesus macaques is also shown (C).

The consequence of reduced pIgR 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 pIgR
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.

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