Persistence of Gut Mucosal Innate Immune Defenses by Enteric α-Defensin Expression in the Simian Immunodeficiency Virus Model of AIDS

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Gastrointestinal (GI) complications, including diarrhea, weight loss, and enteric opportunistic infections, occur frequently in therapy-naive HIV-infected patients (1, 2). HIV infection causes severe CD4+ T cell depletion in gut-associated lymphoid tissue (GALT) early in infection, which contributes to impaired T cell responses against pathogens at gut mucosal sites (3). Studies on HIV pathogenesis have been focused on the impairment of HIV-specific immune response and its role in the development of AIDS (4, 5). However, innate immune defenses play a critical role in the protection of GALT against pathogens, and impairment of the innate immune system may accelerate disease progression. A better understanding of the mucosal innate defenses during HIV infection will provide insights into the mechanisms of HIV enteropathogenesis.

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Melinda M. Zaragoza,* Sumathi Sankaran-Walters,* Don R. Canfield,† Jason K. S. Hung,* Enrique Martinez,* André J. Ouellette,‡ and Satya Dandekar*

Gastrointestinal mucosa is an early target of HIV and a site of viral replication and severe CD4+ T cell depletion. However, effects of HIV infection on gut mucosal innate immune defense have not been fully investigated. Intestinal Paneth cell-derived α-defensins constitute an integral part of the gut mucosal innate defense against microbial pathogens. Using the SIV-infected rhesus macaque model of AIDS, we examined the level of expression of rhesus enteric α-defensins (REDS) in the jejunal mucosa of rhesus macaques during all stages of SIV infection using real-time PCR, in situ hybridization, and immunohistochemistry. An increased expression of RED mRNAs was found in PC at the base of the crypts in jejunum at all stages of SIV infection as compared with uninfected controls. This increase correlated with active viral replication in gut-associated lymphoid tissue. Loss of RED protein accumulation in PC was seen in animals with simian AIDS. This was associated with the loss of secretory granules in PC, suggesting an increase in degranulation during advanced SIV disease. The α-defensin-mediated innate mucosal immunity was maintained in PC throughout the course of SIV infection despite the mucosal CD4+ T cell depletion. The loss of RED protein accumulation and secretion was associated with an increased incidence of opportunistic enteric infections and disease progression. Our findings suggest that local innate immune defense exerted by PC-derived defensins contributes to the protection of gut mucosa from opportunistic infections during the course of SIV infection. The Journal of Immunology, 2011, 186: 1589–1597.
marked reduction in RED protein levels was observed in gut mucosa of animals with advanced SIV disease, coinciding with increased incidence of opportunistic infections. Our findings highlight the importance of α-defensins in protection against mucosal pathogens despite severe CD4+ T cell depletion.

Materials and Methods

Rhesus macaques, SIV infection, and therapy

Peripheral blood and intestinal tissue samples from 44 colony-bred rhesus monkeys (Macaca mulatta) were evaluated (17). Animals were housed in accordance with American Association for Accreditation of Laboratory Animal Care guidelines. Samples from six healthy, SIV-negative animals served as negative controls. Thirty-eight animals were i.v. inoculated with 10–100 animal infectious doses of pathogenic SIVmac251 and were euthanized during primary SIV infection (n = 13), chronic infection (n = 9), or advanced infection with simian AIDS (SAIDS; n = 9). Nine SIV-infected animals received 9-(2R,3R)-[(1,2)-diaminocyclohexyl]propenyl]amine (tenofovir disoproxil fumarate or PMPA; N. Bischoffberger, Gilead) monotherapy once daily at 10–30 mg/kg body weight, for 6–132 wk, beginning at 6 (n = 5) or 10 (n = 4) wk post-SIV inoculation (18). Animals receiving ART were euthanized between 12 and 142 wk postinfection (6–132 wk posttherapy). Longitudinal jejunal biopsy samples were collected by upper endoscopy and peripheral blood samples by venipuncture (19).

Microbiological and parasitological examination of intestinal mucosa

Intestinal specimens collected prior to SIV infection, at the onset of an episode of diarrhea and at necropsy, were examined for the presence of Salmonella, Shigella, Yersinia, Pleisomonas, Aeromonas, and Campylobacter species. Fecal specimens were screened for the presence of parasites and bacterial overgrowth using both a saline and an iodine wet preparation. The formalin/ethyl acetate sedimentation procedure was used for the detection of OVA and parasites. The detection of Cryptosporidium oocysts and Giardia cysts was performed by direct immunofluorescence assay using the Merifluor Crypto and Giardia assay (Meridian Bioscience, Cincinnati, OH). Lesions and occult opportunistic infections were identified by microscopic examination of jejunum tissue sections stained with H&E. Clinical findings were obtained from health records at the California National Primate Research Center.

Measurement of viral burden

Viral burden in plasma and intestinal tissue samples was determined by real time RT-PCR analysis, as previously described (20). A change in the SIV loads was presented as n-fold difference compared with the lowest level of expression, as previously described (ΔΔ cycle threshold method) (20). The SIV-infected cells in intestinal tissue samples were detected and localized using in situ hybridization (ISH) assay (16). Formalin-fixed paraffin-embedded tissue sections were hybridized with digoxigenin (DIG)-labeled SIV gag-specific RNA probe (SIV250, clone from C. Brown, catalogue L3-21005P, Lofstrand Labs, Gaithersburg, MD) at 56˚C for 15 h and processed. SIV-infected cells, stained with NBT-5-bromo-4-chloro-3-indolylphosphate (Roche) in Levamisole and counterstained with nuclear fast red, were visualized under light microscopy. The RED protein-expressing cells with red-brown color were visualized under light microscope. The percentage of crypts with HDS protein was also analyzed.

Statistical analyses

Statistical analysis was performed to determine the significance of the effects of SIV infection or therapy on plasma and gut viral loads, PC counts, and RED expression using one-way ANOVA, followed by Bonferroni’s multiple comparison test (GraphPad Prism version 5.00; GraphPad Software, San Diego, CA; http://www.graphpad.com). When a Levene test was indicated, a weighted least squares was used, in which the weights were chosen to be inversely proportional to the within-group variance. Magnitudes of effects were estimated with back-transformed least squares means.

Results

SIV replication and histopathologic changes in gut mucosa

Thirty-eight SIV infected rhesus macaques were evaluated for the presence of SIV-infected cells and pathologic changes in gut mucosa compared with six uninfected healthy rhesus macaques. SIV-negative animal showed no obvious GI pathology or clinical signs (Table I). Animals with primary or chronic SIV infection exhibited lymphohfollicular hyperplasia or lymphadenopathy and splenomegaly, but had no evidence of pathologic changes or gastri c dysfunction (Table I). Animals with SAIDS exhibited multiple GI complications, including gastrointestinalcolitis, nonspecific diarrhea, and cholecystitis, as well as dehydration, anemia, and pulmonary complications. One SIV-infected animal with rapid disease course (rapid progressor [RP]) developed SAIDS at 12 wk postinfection and had GI complications. A long-term SIV-infected nonprogressor (LTNP) animal suffered from Pneumosyssius simicola infection of the lungs and anemia at the time of necropsy, but experienced only mild GI dysfunction.

Viral burden in gut tissue of SIV-infected animals was evaluated by ISH as well as by real-time RT-PCR (Table I). SIV RNA was detected in lymphocytes and macrophages in the gut mucosa and was localized to the villus tips and cryptopatches (isolated lymphocytic aggregations). A few infected cells were detected in lamina propria surrounding the base of the crypts (Figs. 1, 2). SIV+ cells were not frequent in the villus epithelium, with the exception of animal 26333, which had the majority of its infected cells located in the epithelial layer. Highest numbers of infected cells were seen during primary SIV infection (ISH score = 0–6, median 3.0) or during SAIDS (ISH score = 0–6, median 3.4). Low levels of SIV RNA+ cells were observed in the gut mucosa of therapy-naive animals with chronic SIV infection, as well as in the LTNP (ISH score = 0–2, median 1.3). In contrast, the RP animal had high numbers of SIV-infected jejunal lymphocytes (ISH score = 6).

Viral burden in jejunal mucosa from 26 animals was measured by real-time RT-PCR. In concordance with the ISH findings, highest levels of SIV burden were found during primary and advanced stages of disease, whereas a reduction in the viral burden was noted.
Table I. Summary of clinical and pathological characteristics and microbial findings in study groups

<table>
<thead>
<tr>
<th>Clinicopathological markers</th>
<th>SIV Negative</th>
<th>Primary</th>
<th>Chronic</th>
<th>SAIDS</th>
<th>PMPA Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length of infection (wk)</strong></td>
<td>Uninfected</td>
<td>0.4–2</td>
<td>4–26</td>
<td>11–78</td>
<td>12–142</td>
</tr>
<tr>
<td><strong>Length of therapy (wk)</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>4–132</td>
</tr>
<tr>
<td><strong>Age range (mo)</strong></td>
<td>59–103</td>
<td>32–72</td>
<td>25–68</td>
<td>25–75</td>
<td>82–103</td>
</tr>
<tr>
<td><strong>Weight range (kg)</strong></td>
<td>6.3–8.2</td>
<td>2.7–7.1</td>
<td>2.8–4.4</td>
<td>2.5–6.2</td>
<td>4.6–5.1</td>
</tr>
<tr>
<td><strong>Relative fold Δ in RED</strong></td>
<td>(1.1)–0.9</td>
<td>(1.1)–7.0</td>
<td>1.4–6.8</td>
<td>1.3–1.14</td>
<td>(3.2)–(0.5)</td>
</tr>
<tr>
<td><strong>Viral burden: relative fold Δ(ISH)</strong></td>
<td>UD(0)</td>
<td>$5.3 \times 10^{-4}$–$1.8 \times 10^3/(1–6)$</td>
<td>$1.0 \times 10^4$–$10^5/(0–3)$</td>
<td>UD: $3.0 \times 10^3/(0–6)$</td>
<td>UD: $0.4 \times 10^2/(0–1)$</td>
</tr>
</tbody>
</table>

**Clinical observations**

- **Various commensal bacteria**
  - Lymphofollicular hyperplasia, recurrent diarrhea, various commensal bacteria

- **LA, SM, Pn**
  - Nonresponsive recurrent diarrhea, cachexia, LA, SM, choledocho-cystitis, I/D, pulmonary acariasis, Pn, GEC, various commensal and pathogenic bacteria, anemia

- **L. renale tubular karyomegaly, SM, choledocho-cystitis; I/D, GEC**

**Intestinal pathologic changes**

- **Mild Lp, Id**
  - E: within normal limits
- **Mild Lp, Id**
  - E: acute to chronic, diffuse, mild to moderate
- **Mild to moderate Lp, Id, Ex, vbf**
  - E: subacute to chronic, diffuse, mild to moderate
- **Severe Lp, Id, Ex, vbf**
  - E: subacute to granulomatous, diffuse, mild to severe
- **Mild to moderate Lp, Id, Ex, vbf**
  - E: subacute, diffuse, mild

**Comorbidity factors (%)**

- **Diarrhea**
  - 33
- **Cachexia**
  - 0
- **Lymphadenopathy**
  - 0
- **Splenomegaly**
  - 0
- **Enteritis**
  - 17
- **Colitis**
  - 0

**Secondary infections (%)**

- **Trichomonas hominis**
  - 0
- **Blastocystis hominis**
  - 0
- **Campylobacter (coli or jejuni)**
  - 0
- **Entamoeba coli**
  - 8
- **α-hemolytic Streptococci**
  - 0
- **Balantidium coli**
  - 0
- **Coagulase + Staphylococcus**
  - 0
- **Cryptosporidia**
  - 0
- **Giardia**
  - 0
- **Gram – rods, anaerobic**
  - 0
- **Streptococcus viridans**
  - 0

**Bold font designates values that differ significantly from other treatment groups ($p < 0.0395$).**

**Determinations made by veterinary pathologists at the time of necropsy.**

**Determinations made by retrospective examination of formalin-fixed tissues.**

**Data for comorbidity factors and secondary infections were obtained directly from clinical records maintained at the California National Primate Research Center (Davis, CA). Microbial and parasitological examinations performed within 1 mo of necropsy were included within these estimations.**

E, enteritis; Ex, eosinophils; GEC, gastroenterocolitis; I/D, inanition/dehydration; Id, lacteal dilatation; LA, lymphadenopathy; Lp, lymphopenia; NA, data are not available for this group; Pn, pneumonia; SM, splenomegaly; UD, undetectable levels (below 50 copies) of SIV RNA; vbf, villus blunting/fusion.
during chronic SIV infection (Fig. 3, Table I). Analysis of plasma samples from 17 animals by real-time RT-PCR showed that plasma viral loads correlated with tissue viral burden, with the highest levels seen during primary and SAIDS stages of infection and decreased burden during chronic SIV infection and ART (Supplemental Fig. 1). As previously demonstrated, CD4+ T cell depletion was observed in peripheral blood and was dramatically evident in GALT (Supplemental Fig. 1) (21).

**Increased expression of RED and PC numbers in response to SIV infection**

The location of RED mRNA-expressing cells, performed by ISH, was restricted to the base of crypts, and they were identified morphologically as PC based on presence of apical granules, basal nucleus, and pyramidal shape (Fig. 4A (22)). Longitudinal analysis of jejunal biopsy samples from six SIV-infected animals detected expression of RED mRNAs throughout the course of SIV infection (Fig. 4B). Our results indicated that RED mRNA was expressed in ∼98% of crypts in the jejunal mucosa of healthy uninfected animals and did not significantly change through the course of SIV infection (Fig. 5F). Variation in the intensity of RED immunoreactivity did not correlate with stage of disease or viral burden.

Quantification of RED mRNA levels in gut mucosa demonstrated an increased expression of RED transcripts in SIV-infected, therapy-naive animals during primary (mean 2.8-fold increase), chronic (mean 3.7-fold increase), or SAIDS (mean 4.7-fold increase) stages of infection, as compared with uninfected controls (Fig. 3). A significant increase in RED expression was seen in SIV-infected, therapy-naive macaques as compared with uninfected controls and PMPA-treated animals (*p*, 0.04; Fig. 3), and was associated with more PC per jejunal crypt (Fig. 5D). Examination of H&E-stained gut tissue sections detected an average of 5 PC per crypt in SIV-negative controls, whereas SIV-infected animals had 10, 12, and 14 PC per crypt during primary, chronic, and SAIDS stages of infection, respectively. Collectively, our data suggested that SIV infection led to an increase in RED gene expression and expansion of PC numbers in jejunal mucosa that remained elevated throughout the course of SIV infection, independent of the magnitude of viral burden within the gut mucosal tissue.
Decreased RED protein levels correlate with enteric opportunistic infections and advanced SIV disease

IHC analysis was performed to determine the cellular localization of RED proteins. RED proteins were detected in jejunal tissues from uninfected and SIV-infected animals during the primary and chronic stages of infection, as well as in the LTNP animal (92% RED-positive crypts) (Fig. 6E–H). IHC analysis showed a reduction of RED protein levels in PC in SAIDS animals, including the animal with rapid disease progression (Fig. 6G, 6H). The percentage of RED⁺ crypts did not differ between SIV-ve control animals and SIV⁺ asymptomatic animals. However, a sharp decline in the percentage of RED⁺ crypts was observed in animals with SAIDS (Fig. 5E).

Although the primary structure of HD-5 differs greatly from known RED peptide sequences, a short region of highly conserved sequence identity exists between RED4, RED8, and HD5, shown as underlined, bold residues in the HD-5 sequence: ATCYCRTGRCATRESLSG VCEISGRLYR LCCR. The shared CRTGRC could be an epitope shared by these REDs that is recognized by anti–HD-5 Ab. The defensin immunoreactivity in the lumen appeared to be increased in the animals with SAIDS, as compared with gut tissues from SIV-negative control animals, and in animals with primary or chronic SIV infection, or SIV-infected animals receiving ART. The number of PC in the crypts of animals with SAIDS remained unchanged (Fig. 5D). There was no corresponding immunoreactivity in the Ab-negative controls, suggesting that defensin immunoreactivity might represent defensin protein in lumen (Fig. 6G, 6H). Mucosal tissue was also evaluated for the presence of granules to determine the extent of degranulation in the PC during the course of SIV infection. Very few eosinophilic granules could be detected in PC in jejunal tissue samples from SAIDS animals as compared with uninfected controls and animals during primary or chronic SIV infection (Figs. 5E, 6).

Serial gut tissue sections examined for either RED mRNA or protein, or SIV RNA demonstrated that RED protein was localized to dense granules and RED mRNA to the cytoplasm of corresponding cells in tissues from all animals. SIV RNA was not detected in PC, suggesting that PC did not support viral replication. A representative example of RED expression in an SIV-infected animal during primary acute stage of infection with high viral burden is shown (Fig. 5).

Advanced SIV disease was marked by a significant loss of RED protein in PC that coincided with an increase in the incidence of bacterial and protozoal infections (Table I). The infections included Fusobacterium, Bacteroides, α-hemolytic Streptococcus, Streptococcus viridans, coagulase-negative/positive Staphylococci, Corynebacterium, Pseudomonas maltophilia, Iodamoeba butschlii, Peptostreptococcus, and Lactobacillus species, each of which was found in one or more animals. Pentatrichomonas hominis was the...
most prevalent pathogen in SAIDS animals (seven of seven) despite treatment of five of these animals with metronidazole. There was a significant association with the loss of RED protein and the presence of *Trichomonas* infection of the large intestine in animals with SAIDS (*p* ≤ 0.0004). *Blastocystis hominis* was the second most common infection (five of seven). This may have been due to the ineffectiveness of most antiprotozoal medications against the organism. *Entamoeba coli* and *Campylobacter* were the third most prevalent pathogens in SAIDS animals (four of seven). *E. coli* are typically nonpathogenic, even in people with impaired immune functions. However, a breakdown of mucosal epithelium may enable its entry and colonization. Animals with SAIDS, including the RP animal, had 8 or more secondary infections and had nonresponsive, recurrent diarrhea even after metronidazole therapy. Two of these animals had high viral burden (ISH score = 5), but one animal had a very low viral burden (ISH score = 1).

Two animals with primary SIV infection had three or more secondary infections. The presence of RED protein in the PC of these animals was similar to that seen in uninfected animals. A high incidence of *Campylobacter jejuni* or *C. coli* was seen during the primary acute stage of infection, but was not evident during chronic SIV infection. Specific immunity, involving intestinal Ig (IgA) and systemic Abs, is required for healthy animals to control *Campylobacter* infection. The proinflammatory environment and eventual breakdown of the mucosal epithelial barrier during SAIDS could predispose animals to recurrence of *Campylobacter* infection due to entry and colonization.

**ART led to suppression of SIV replication and maintenance of enteric defensin expression**

We evaluated the effect of ART on viral replication and expression of RED peptides during SIV infection. The ART led to suppression of SIV loads in peripheral blood and in gut mucosal tissues (Table I). IHC and ISH analyses detected the expression of RED mRNA and protein in gut tissue samples from all SIV-infected animals receiving ART (Fig. 7). However, quantitative analysis of RED mRNA expression by real-time PCR showed that animals receiving ART had significantly decreased RED mRNA levels compared with therapy-naive SIV-infected animals, but similar to SIV-negative control animals (1.6-fold decrease, *p* < 0.0003) (Fig. 3). Our data showed that viral suppression induced by ART of SIV-infected monkeys might lead to the maintenance of RED protein expression that is comparable to uninfected healthy controls and better protection against enteric microbial infections. Thus, prevention of the loss of RED expression in the gut mucosa of these animals during ART would contribute to effective innate immunity at the mucosal site.

**Discussion**

One of the most common causes of mortality among ART-naive, HIV-infected individuals is the incidence of opportunistic infections. The innate immunity plays an important role not only during the initial stages of HIV infection, prior to the development of a virus-specific adaptive immune response, but also throughout the course of infection by protecting the host against various pathogens. Our study examined the role of mucosal innate defense in HIV infection by analyzing changes in the expression of rhesus enteric α-defensins in gut mucosa of SIV-infected rhesus macaques in the context of viral replication and CD4+ T cell depletion. Our findings showed an increased level of RED gene expression associated with an increase in PC numbers at all stages of SIV infection in therapy-naive SIV-infected animals. This suggests that REDs may be an important component of the gut mucosal innate immune defense that persists through primary and chronic stages of SIV infection.
Several mechanisms may contribute to the upregulation of REDs in the PC during SIV infection and their loss during the advanced stages of infection. Severe loss of CD4+ T cells including CD4+ Th17 cells in gut mucosa during primary HIV and SIV infections has been well documented (15, 23). This CD4+ T cell loss is not reversed despite the development of virus-specific humoral and cellular responses (3). The immediate and long-term impact of mucosal CD4+ T cells is not fully defined. It has been demonstrated that SIV-induced loss of CD4+ Th17 T cell in gut mucosa of SIV-infected rhesus macaques led to impaired Th17 CD4+ T cell responses to the Salmonella typhimurium infection in the gut mucosa and resulted in increased systemic bacterial translocation (24). Thus, the gut mucosal CD4+ T cell depletion may lead to incomplete control of enteric pathogens and increased microbial translocation from the lumen. An increase in RED gene transcription may be a mechanism by which the host mucosal response contains the microbial burden. It is also reasonable to propose that increased RED expression may partly compensate for the immune defects generated in the gut mucosa by SIV infection. Whereas the number of REDs in rhesus macaque PC far outnumbers that in human PC, identification of individual defensins is beyond the scope of this study. Their antimicrobial and antiviral activity may be crucial in providing protection to crypt epithelial progenitor cells as well as T cells in the neighboring regions. Further characterization of PC-derived RED peptides will be important to gain better understanding of the PC-mediated mucosal innate defense.

Increased microbial translocation due to the breakdown of the gut epithelial barrier in SIV infection may also impact enteric defensin gene expression and peptide levels and warrants further investigation. Disruption of the integrity of the intestinal epithelium in HIV and SIV infections has been well documented and is linked to decreased nutrient digestion and malabsorption and increased microbial translocation (24, 25). Increased expression of genes associated with inflammation and immune activation in the gut mucosa during HIV and SIV infections has been reported (21). Histopathologic changes of villus atrophy and crypt hyperplasia along with the mucosal CD4+ T cell loss may contribute to mucosal inflammation and inability to control microbial burden. Our findings of increased PC numbers in the gut mucosa suggest that the impairment or loss of adaptive immune responses and the integrity of the villus epithelial barrier secondary to SIV infection may result in the need for a compensatory increase in crypt innate responses resulting in expansion of the PC compartment. In a mouse model of inflammatory bowel disease, gut mucosal inflammation and disruption of PC homeostasis with hyperplasia have been reported that were associated depletion of PC gene products and secretory granules (26). ART is effective in suppressing viral loads and reducing inflammation in the gut mucosa. We found that animals receiving ART had RED expression comparable to the uninfected controls and low to almost no incidence of opportunistic infections in the GI tract as compared with untreated animals, indicative of better mucosal immune functions (27). It is possible that this may reduce PC secretion of innate proteins, thus...
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preventing the exhaustion of this mucosal innate immune response. Previous studies have demonstrated that interruption of ART in SIV-infected animals led to a rapid loss of GALT CD4+ T cells and increased viral loads similar to that seen in primary SIV infection (28). Whereas our study was not designed to investigate these effects, it suggests that therapy interruption may result in increased opportunistic infections as seen in untreated SIV infection, resulting in increased RED expression following decreased degranulation.

We found a loss of intestinal PC granules and detection of RED protein in SIV-infected animals with SAIDS, which was accompanied by increased immunostaining in the lumen of jejunal tissues, suggesting high levels of secretion of REDs into lumen (Supplemental Fig. 2). Loss of PC defensin expression through increased degranulation was previously observed in the small intestine of HIV-infected patients with AIDS (29). The loss of defensins in PC during SAIDS may be attributed to the release of the granules. Furthermore, the loss of RED protein in animals with SAIDS did not correlate with viral burden, but rather with the advanced stage of disease. An animal that had a rapid SIV disease course of 12 wk showed a loss of RED protein expression. Increased bacterial burden may cause rapid degranulation and loss of RED protein from PC in advanced SIV infection. Increased bacterial burden may cause rapid degranulation and loss of RED protein from PC in advanced SIV infection. It is also possible that the RED peptide biosynthesis may not sustain the loss of RED protein due to the increased bacterial load in the gut mucosa. A strong association between the loss of RED protein and incidence of Trichomonas infection in the large intestine was noted. Studies have shown that α-defensins are variably effective against eukaryotic pathogens, depending on their primary structures, and may have shown that a strong association between the loss of RED protein and incidence of opportunistic infections as seen in untreated SIV infection, resulting in increased RED expression following increased degranulation.

In summary, the loss of enteric defensins in SAIDS is associated with physiological exhaustion of the regulated biosynthetic secretion pathway of PC, which may be prevented by viral suppression and immunomodulatory therapies. Future studies are needed to determine the mechanism of the loss of REDs in SAIDS, as well as how this loss affects disease progression. Our study showed that PC-derived enteric defensins may play an important role in mucosal defense by providing antimicrobial functions and protecting the gut mucosa against infections. Thus, further studies of α-defensins may help develop strategies to exploit innate defense molecules for effective control of secondary enteric infections in HIV-infected patients.

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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Figure 1: Plasma Viral loads, Peripheral Blood CD4+ T cell counts and Jejunal Mucosa CD4+ T cell % in SIV infected animals and SIV negative controls. (A) Animals in the chronic stage of SIV infection had lower viral loads than during the primary stage (set point). PMPA significantly reduces viral loads in infected macaques. (B) SIV infection reduces CD4+ T cell numbers in peripheral blood. (C) A dramatic loss of CD4+ T cells was observed in all stages of infection compared to uninfected controls. Incomplete CD4+ T cell restoration observed in both PBMC and LPL. * indicated significant differences between groups.
Supplemental Figure 2. Model of rhesus enteric α-defensin alterations in SIV-infection.

**SIV Uninfected**
- Normal RED mRNA levels
- RED in PC Granules

**SIV (+) Asymptomatic Infection**
- Increased RED mRNA levels
- RED in PC Granules

**SIV (+) SAIDS**
- Increased RED mRNA levels
- Degranulation: RED not in PC Granules; RED in lumen