HIV Infection in Gastric Epithelial Cells

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Many chronic human immunodeficiency virus (HIV) patients suffer from gastric complaints, including gastric tuberculosis and coinfection of other pathogens. Recent work has demonstrated that a variety of nonimmune cells can act as viral reservoirs, even at the early stage of HIV infection. In this study, we detect HIV viral particles, proteins, and nucleic acids in gastric epithelial cells using clinical samples. These observations are further supported by a simian immunodeficiency virus–infected macaque model. Further, the number of HIV-infected gastric epithelial cells is positively associated with blood viral load, and is negatively correlated with CD4 lymphocyte cell counts. We also demonstrate that HIV infection is accompanied by severe inflammatory response in gastric mucosa. Additionally, HIV infection activates signal transducer and activator of transcription 3 and RelA, and enhances the production of interleukin 6 and tumor necrosis factor α in gastric epithelial cells. The present data suggest that the gastric epithelial cells are natural targets of HIV infection, and HIV infection in epithelial cells contributes to HIV-induced gastric mucosal inflammation.

Keywords. AIDS; epithelial cells; gastric mucosa; HIV.

Even though the use of highly active antiretroviral therapy (HAART) has reduced the global incidence of acquired immunodeficiency syndrome (AIDS) in the past 2 decades by reducing the viral load in human immunodeficiency virus (HIV)–infected patients, HIV/AIDS remains a major global health concern [1]. It is well established that loss of CD4 lymphocytes in peripheral blood is one of the hallmarks of AIDS progression [2]. In addition to peripheral blood, gastrointestinal mucosal-associated lymphoid tissue (MALT) is known to be a target for early HIV infection, and is a site of severe CD4 lymphocyte depletion [3]. It has been reported that the majority of CD4 lymphocytes resident in gastrointestinal MALT are rapidly depleted during acute HIV infection [4]. In contrast to peripheral blood, there is a significant delay in the restoration of CD4 lymphocytes in intestine mucosa, even following HAART [5].

In addition to CD4 lymphocytes, HIV can infect a variety of nonimmune cells, including microglia, astrocytes, and fibroblasts, which act as HIV reservoirs, even at the early stage of HIV infection [6–8]. Notably, opportunistic HIV infections in epithelial cells have been recently reported. These studies have demonstrated the presence of HIV DNA, RNA, and protein antigens in intestinal [9, 10] or renal epithelial cells [11] from clinical specimens. Importantly, HIV infection in these epithelial cells leads to mucosal dysfunction, and is thought to be involved in HIV-associated diseases, including HIV-associated diarrhea [12] and HIV-associated nephropathy (HIVAN) [13].

It has been reported that many HIV-infected patients with upper gastrointestinal symptoms displayed gastropathy by biopsies, including gastric tuberculosis, gastric hypoacidity, and coinfection of other pathogens [14]. However, our knowledge on HIV infection in gastric mucosa and HIV-associated gastric pathology is very limited. In this study, we present novel findings showing that HIV infects gastric epithelial cells, which was involved in HIV-mediated mucosal inflammation.
MATERIALS AND METHODS

Clinical Samples
A total of 136 gastric mucosa tissues from HIV-infected patients were collected and used in this study, including 59 biopsy tissues from asymptomatic HIV-infected patients, 75 biopsy tissues from AIDS patients, and 2 autopsy tissues from AIDS cadavers. Of these, 50 asymptomatic samples, 54 AIDS samples, and 1 autopsy sample were collected from Sichuan Center For Disease Prevention and Control (Chengdu, China); 9 asymptomatic samples and 21 AIDS samples were collected from Beijing Youan Hospital, Capital Medical University (Beijing, China); 1 autopsy sample was collected from the 302 Hospital of PLA (Beijing). The gastroscopic samples from non-HIV superficial gastritis patients were collected from West China Hospital, Sichuan University (Chengdu), and used as non-HIV controls. After gastroscopic excision, the clinical samples (HIV-infected and non-HIV infected) were immediately fixed in formalin for immunostaining, or fixed in 4% paraformaldehyde containing 0.1% glutaraldehyde for transmission electron microscopy (TEM) analysis. The Institutional Ethics Committee of Sichuan University approved this study, and informed consents were obtained from all patients prior to analysis.

Transmission Electron Microscopy
Tissue samples were fixed in 4% paraformaldehyde containing 0.1% glutaraldehyde for 8 hours, and then stained with 3% aqueous uranyl acetate for 1 hour. After rinsing with water, the samples were dehydrated in an increasing gradient of alcohol (50%, 75%, and 95%–100%), and finally embedded in Epon-Araldite resin (Canemco, 034). Ultrathin sections were prepared using a Reichert ultramicrotome, and analyzed using a transmission electron microscope (Philips EM420).

CD4 Lymphocyte Count
Well-mixed, anticoagulated whole blood was transferred into Trucount tubes, which were purchased from BD Biosciences (San Jose, CA). The blood cells were then labeled with PerCP-conjugated anti-CD3 and APC-conjugated anti-CD4 (BD PharMingen, San Diego, CA) for 30 minutes. Lysing solution was added into the tube for lysing red blood cells. After incubation for 15 minutes in the dark at room temperature, flow cytometric analyses were performed using a FACScalibur with CELLQuest software (Becton Dickinson, San Jose, CA). Those cells showing both CD3 and CD4 signals were selected as CD4 lymphocyte.

Measurement of HIV Viral Load
The 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) was used to measure viral load in peripheral blood from patients, as previously reported [15].

For measuring total HIV viral load in mucosa, total RNA from gastric mucosa tissues was extracted and purified using a RNeasy kit (Qiagen, Germantown, MD). The quantitation of HIV RNA was carried out by using the Cobas Amplicor HIV-1 Monitor Test, version 1.5 (MCA; Roche Molecular Systems, Inc, Branchburg, NJ), a previously described commercial semi-automated real-time polymerase chain reaction assay [16].

Establishment of Simian Immunodeficiency Virus–Infected Macaque Model
Five macaques were infected with simian immunodeficiency virus (SIV) mac239 to establish an SIV infection model. Macaques (Macaca mulatta) were housed in accordance with the American Association for Accreditation of Laboratory Animal Care standards. Infection was initiated by intravenous infusion of 5 × 10^3 TCID50 (50% tissue culture infectious doses) of SIVmac239. Two macaques were randomly selected to examine SIV infection in the gastric mucosa by immunostaining. Macaque gastric mucosa samples were obtained immediately after an SIV-infected macaque died due to SIV/AIDS complication. Occurrence of SIV infection in gastric epithelial cell in a tissue sample was defined as positive when at least 5 p27- or Nef-positive epithelial cells were found in this sample. Gastric mucosa tissues from 2 uninfected macaques was used as control groups.

In situ Hybridization
In situ hybridization was performed using 2 gag gene probes for HIV gene detection. The sequences were as follows: probe 1,5′ ATC CTG GGA TAA AAT AAA ATA GTA AGA ATG TAT AGC CCT AC 3′; probe 2, 5′ CAC CAT GGT AAA TAC AGT AGG GGG ACA TCA AGC AGC CAT GCA 3′. Two distinct methods of in situ hybridization were used to ensure the reliability of experimental results. Briefly, in the first method using probe 1, formalin-fixed, paraffin-embedded tissue sections were soaked with 0.01% diethylpyrocarbonate (DEPC) H2O, digested with proteinase K, followed by triethanolamine and acetic anhydride. Then, the sections were destructured, prehybridized, and incubated with a biotin-labeled probe in a hybridization cocktail plus dextran sulfate in formamide, and hybridized at 47°C overnight. After hybridization, the sections were washed with gradient saline-sodium citrate, labeled with alkaline phosphatase, stained, and examined by light microscopy. In addition, a commercial kit (Taipu, Fuzhou, China) for in situ hybridization was employed to detect HIV with probe 2, following the protocol described by the manufacturer. Briefly, formalin-fixed, paraffin-embedded tissue sections were soaked with 0.01% DEPC H2O, and treated with hybridization-reinforcing agent and peroxidase-blocking agent. Then, the sections were destructured, prehybridized, and hybridized at 37°C overnight. After hybridization, the sections were incubated with antibody, stained, and examined by light microscopy. The biopsy tissues obtained from non-HIV gastritis patients and labeled with the antisense probe, or the samples obtained from HIV-infected patients and labeled with the sense probe were
used as controls. Occurrence of HIV infection in gastric epithelial cell in a tissue sample was defined as positive when at least 5 gag-positive epithelial cells were found in the sample.

**Immunostaining**

Tissues were formalin-fixed and paraffin-embedded, and sections were consecutively cut into 3–4 µm thickness for immunostaining using a Dako EnVision System (DakoCytomation GmbH, Hamburg, Germany) according to the manufacturer’s instructions. Briefly, the paraffin sections were dewaxed, rehydrated, and incubated in 3% H2O2 for 10 minutes in the dark at room temperature to quench the endogenous peroxidase activity. Antigen retrieval was performed in citrate buffer (pH 6.0) using the autoclave sterilizer method. Subsequently, the sections were blocked by normal rabbit or goat serum diluted in phosphate-buffered saline (PBS; pH 7.4) for 20 minutes at 37°C, followed by an incubation at 4°C overnight with the following primary antibodies: anti-CD3 (diluted 1:150, Santa Cruz), anti-CD8 (diluted 1:100, Santa Cruz), anti-CD38 (diluted 1:200, Abcam), anti-CD57 (diluted 1:100, Santa Cruz), anti–tumor necrosis factor (TNF) α (diluted 1:100, Abcam), anti-SIV p27 (diluted 1:500, eENZYME), anti-SIV Nef (diluted 1:100, Abcam), anti-E-cadherin (diluted 1:100, Abcam), anti-pan-Keratin (diluted 1:200, Abcam) anti-HIV p24 (diluted 1:200, Millipore; diluted

Figure 1. Detection of HIV infection in gastric epithelial cells by in situ hybridization and immunostaining. A, p24 (upper panel) and HIV gag (bottom panel) were detected in lymphocytes gathering in folliculus lymphaticus by immunostaining or in situ hybridization, respectively. B, HIV gag was detected in gastric epithelial cells from HIV-infected patients by in situ hybridization using 2 different probes. The samples obtained from non-HIV gastritis patients and labeled with the antisense probe, or the samples obtained from HIV-infected patients and labeled with the sense probe were used as controls. C, HIV p24 protein was detected in the gastric epithelial cells from HIV-infected patients by immunostaining using 3 different p24 antibodies. The samples obtained from non-HIV gastritis patients and probed with p24 antibody, or the samples obtained from HIV-infected patients and probed with normal IgG were used as controls. Antibody 1, Abcam (ab53841); antibody 2, Millipore (MAB880-A); antibody 3, Santa Cruz Biotechnology (sc-69726). Abbreviations: HIV, human immunodeficiency virus; IgG, immunoglobin G.
and careful discussion until agreement was reached. The score for each slide was measured as the cross product of the value of immunostaining intensity (A) and the value of proportion of staining-positive cells (B) from 10 randomly selected areas, as described previously [17, 18]. Immunostaining intensity was divided into 4 grades: 0, negative; 1, weak; 2, moderate; and 3, strong. The proportion of staining-positive cells was divided into 5 grades: 0, <5%; 1, 6%–25%; 2, 26%–50%; 3, 51%–75%; and 4, >75%. The results were defined as negative (−, 0), positive (+, 1–6), or strong positive (+++, 8–12). Slides were scored and confirmed by 2 separate, experienced pathologists who were blinded to all patient information, including HIV infection and clinical status. Any discrepancy between the 2 evaluators was resolved by reevaluation by a third pathologist and careful discussion until agreement was reached.

For immunofluorescence staining, after incubation of primary antibodies, the slides were rinsed in fresh PBS 3 times, and incubated with secondary antibody conjugated to fluorescein isothiocyanate or tetramethyl rhodamine isothiocyanate for 30 minutes at room temperature. The specimens were analyzed using a Zeiss Stemi 2000-C fluorescence microscope (Carl Zeiss Microimaging Inc) or an FV1000 confocal microscope (Olympus).

For examination of HIV infection in gastric epithelial cells, biopsy tissues obtained from non-HIV gastritis patients and probed with p24 antibody, or the tissues obtained from HIV-infected patients and probed with normal immunoglobulin G were used as controls. Occurrence of HIV infection in gastric epithelial cell in a tissue sample was defined as positive when at least 5 p24-positive epithelial cells were found in the sample.

**Data Analysis and Statistics**
Comparisons between 2 groups were performed by Student’s t test. Statistical significance was defined as *P < .05, **P < .01, ***P < .001.

**RESULTS**

**Gastric Epithelial Cells Are the Natural Targets of HIV Infection**
In an initial study, we examined the HIV infection status of gastric mucosa by in situ hybridization and immunostaining. HIV-infected samples, including 59 biopsy tissues from HIV-infected asymptomatic patients, 75 biopsy tissues from AIDS patients, and 2 autopsy tissues from AIDS cadavers, were collected. In addition, 20 biopsy tissues obtained from non-HIV gastritis patients were used as non-HIV control. As expected, HIV infection was detected in lymphocytes which gathered in the folliculus lymphaticus (Figure 1A). Surprisingly, HIV infection could also be observed in gastric epithelial cells. As shown, by in situ hybridization, gastric epithelial cells harboring HIV nucleic acids were also detected in 83.1% (49/59) of the asymptomatic samples and 96.0% (72/75) of the AIDS samples by probe 1, and in 88.1% (52/59) of the asymptomatic samples and 90.7% (68/75) of the AIDS samples by probe 2 (Figure 1B and Table 1). In contrast, similar signals were not observed in non-HIV gastritis tissues by using the antisense probe or in HIV-infected tissues by using the sense probe. Similarly, a positive HIV signal was also observed in gastric epithelial cells using 3 distinct antibodies recognizing HIV p24 protein (Figure 1C). As shown in Table 1, p24-positive epithelial cells were found in 74.6% (44/59) of the asymptomatic samples and 97.3% (73/75) of the AIDS samples with antibody 1, 79.7% (47/59) of the asymptomatic samples and 92.0% (69/75) of the AIDS samples with antibody 2, and 76.3% (45/59) of the asymptomatic samples and 86.7% (65/75) of the AIDS samples with antibody 3. Further, by TEM analysis, HIV particles were observed in the cytoplasm of epithelial cells in gastric tissue from both asymptomatic and AIDS patients (Figure 2A and 2B). To further validate the localization of HIV virus and gastric epithelial cells, immunofluorescence staining was employed by using 2 epithelial cell markers, pan-Keratin and E-cadherin [19, 20], respectively. As shown in Figure 2C, clusters of E-cadherin- or Keratin-positive cells were found to be p24-positive by confocal imaging, indicating that those HIV-infected cells in acini are epithelial cells.

**Table 1. Detection of HIV Infection in Gastric Epithelial Cells by in situ Hybridization and Immunostaining**

<table>
<thead>
<tr>
<th>N</th>
<th>ASYM* (n = 59)</th>
<th>AIDS (n = 75)</th>
<th>Cadavers (n = 2)</th>
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<tr>
<td>Probe 1</td>
<td></td>
<td></td>
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<td>−</td>
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<td>73.3% (55/75)</td>
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</tr>
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<td>0</td>
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<tr>
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</tr>
<tr>
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<td>58.7% (44/75)</td>
<td>100% (2/2)</td>
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</tr>
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<td>0</td>
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<tr>
<td>++</td>
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<td>58.7% (44/75)</td>
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</tr>
<tr>
<td>++</td>
<td>35.6% (21/59)</td>
<td>40% (30/75)</td>
<td>100% (2/2)</td>
</tr>
</tbody>
</table>

Abbreviations: AIDS, acquired immune deficiency syndrome; ASYM, asymptomatic; HIV, human immunodeficiency virus; N, number of HIV-positive epithelial cells in a slide.

* N: −, N < 5; +, 5 ≤ N < 100; ++, 100 ≤ N.
To further support our observation, SIV infection in gastric mucosa was examined by immunostaining for SIV p27 or Nef in 2 SIV-infected macaques. As a result, either p27- or Nef-positive gastric epithelial cells was detected, which was similar to the observation in the human clinical samples (Figure 3A and 3B). Based on these results, we concluded that gastric epithelial cells are the natural targets of HIV infection in gastric mucosa.

**HIV Infection in Gastric Epithelial Cells Is Associated With HIV Viral Load and Abundance of Peripheral CD4 Lymphocytes**

Given that HIV could infect gastric epithelial cells, we set out to determine the correlation of HIV infection in gastric epithelial cells with a series of clinical indices. To avoid the influence of antiretroviral treatment, 66 biopsy samples obtained from the patients who had not received antiretroviral therapy were selected. First, we evaluated the number of HIV-infected epithelial cells in gastric mucosa in relation to the HIV viral load. Statistical analysis showed that the number of HIV-infected epithelial cells in gastric mucosa was significantly higher in the patients with a high viral load in peripheral blood ($y = 0.002x + 4.156$, $R^2 = 0.35$; $P = .0004$) (Figure 4A), suggesting that HIV infection in gastric epithelial cells was positively correlated with blood viral load. However, no significant correlation was found between the number of HIV-infected epithelial cells and mucosal total viral load (data not shown).

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**Figure 2.** Detection of HIV infection in gastric epithelial cells by TEM analysis and immunofluorescence staining. A and B, HIV infection in gastric epithelial cells was detected by TEM analysis using clinical samples at both asymptomatic (A) and AIDS stages (B, both upper and bottom panels). Arrows, HIV particles. C, Coimmunofluorescence staining of p24 and pan-Keratin (upper panel) or p24 and E-cadherin (bottom panel). The slides were visualized by confocal imaging. Abbreviations: N, nucleus; C, cytoplasm; T, tight junction; HIV, human immunodeficiency virus; TEM, transmission electron microscopy.
Next, we analyzed the relationship between the number of p24-positive gastric epithelial cells and the abundance of peripheral CD4 lymphocytes. As shown in Figure 4B, linear regression analysis indicated that the percentage of p24-positive epithelial cells was enhanced while the abundance of peripheral CD4 lymphocytes decreased ($y = -0.0348x + 18.635$, $R^2 = 0.30$, $P < .0001$). As CD4 lymphocyte count is an important criteria for clinical staging of HIV chronic infection [2], the numbers of HIV-infected epithelial cells in asymptomatic and AIDS stages were compared. At the asymptomatic stage, only a small number of epithelial cells with positive p24 signals were found in the lamina propria (Figure 4C, left panel). The percentage of HIV-infected epithelial cells and the percentage of HIV-infected glandular acini (glandular acini containing at least 1 p24-positive epithelial cell) were 5.1% and 13.2%, respectively. At the AIDS stage, the percentage of HIV-infected epithelial cells and the percentage of HIV-infected glandular acini increased to 17.3% ($P < .01$) and 34.2% ($P < .05$), respectively (Figure 4C, middle panel). Notably, both gag and p24 signals were observed in almost all the epithelial cells in autopsy tissues (Figure 4C, right panel). These data indicated that HIV infection in gastric epithelial cells increased at the late stage of chronic infection.

**HIV Infection in Gastric Epithelial Cells Induced an Inflammatory Micromilieu**

As gastric complaints were frequently reported in HIV-infected patients, we next assessed the pathological changes in gastric mucosa under HIV infection. To this end, 31 biopsy tissues from HIV-infected patients who had not received antigastritis treatment were selected. Because healthy gastric mucosal tissue is hard to obtain, 20 biopsy tissues from superficial gastritis patients who had not received antigastritis treatment were collected and used as non-HIV control. As shown in Figure 5A, compared to non-HIV control, most HIV-infected gastric tissues exhibited severe inflammatory cell infiltration, affecting the upper 2/3 or full thickness of mucosa. Notably, a general glandular atrophy was observed in 35.7% (5/14) of asymptomatic patients and 82.4% (14/17) of AIDS patients.

To extend these pathological observations, we examined the abundance of several distinct subtypes of the infiltrating inflammatory cells. It was found that the number of total lymphocytes (CD3$^+$), activated lymphocytes (CD38$^+$), CD8$^+$ lymphocytes, and natural killer cells (CD57$^+$) were generally augmented in the HIV-infected gastric mucosa compared to non-HIV control (Figure 5B–E and Supplementary Table 1). However, no significant difference was observed between the HIV-infected tissues and non-HIV control.
and the non-HIV control with respect to the number of CD20+ lymphocytes (data not shown). These results suggested that, compared to non-HIV superficial gastritis tissues, HIV infection is accompanied with significant inflammatory response in gastric mucosa.

To elucidate the proinflammatory effect of HIV-infected gastric epithelial cells, 2 immune-associated transcription factors, signal transducer and activator of transcription (STAT)–3 and RelA, were used as markers to monitor immune activation in epithelial cells. As shown in Figure 6A, phosphorylation of STAT-3 (Tyr705) and RelA (Ser536) was detected in the p24-positive epithelial cells in clinical samples, suggesting that HIV infection triggered immune activation in the infected epithelial cells. Further, activation of STAT-3 and RelA was also observed in those p24-negative epithelial cells neighboring the p24-positive epithelial cells. Activation of STAT-3 and RelA could initiate the production of a spectrum of inflammatory factors, including TNF-α and interleukin 6 (IL-6) [21, 22]. As shown, either TNF-α or IL-6 expression was found in HIV-infected epithelial cells by confocal imaging (Figure 6B). Further, at the asymptomatic stage, accumulation of TNF-α and IL-6 was detected in p24-positive epithelial cells as well as the cells neighboring the p24-positive epithelial cells, whereas these factors were rarely detected in the p24-negative epithelial cells far away from the infected epithelial cells (Figure 6C, left panel). At the AIDS stage, the number of epithelial cells expressing either of the 2 factors was markedly
elevated compared to the sample from the asymptomatic stage (Figure 6C, right panel), corresponding to the increased abundance of HIV-infected epithelial cells. These data suggested that HIV infection in gastric epithelial cells induces an inflammatory micromilieu in gastric mucosa.

**DISCUSSION**

Accumulating evidence suggests that gastrointestinal MALT is the reservoir for HIV replication [3, 4]. However, little is known about HIV infection in gastric epithelium. In this study, HIV proteins and nucleic acids were detected in gastric epithelial cells in both biopsy and autopsy tissues from HIV-infected patients, and TEM analysis demonstrated the occurrence of HIV viral particles in the cytoplasm of gastric epithelial cells. Further, we examined the status of SIV infection in macaque gastric mucosa at the protein level. Although a previous study failed to identify SIV-infected epithelial cells throughout the gastrointestinal track in a macaque model using in situ hybridization [23], in the present data, we showed that expression of both SIV Nef and p27 was detected in macaque gastric epithelial cells. Therefore, this study provides solid evidences supporting HIV infection in gastric epithelial cells.

In addition, we found that the abundance of HIV-infected epithelial cells in gastric mucosa varied with a series of clinical indices. First, the number of HIV-infected gastric epithelial cells was positively correlated with the blood viral load. Second, the number of HIV-infected gastric epithelial cells was increased with the progression of chronic infection and was markedly elevated at the AIDS stage compared to the asymptomatic stage. Third, the number of HIV-infected gastric epithelial cells was negatively related with the number of CD4 lymphocytes in peripheral blood. Based on these data, it is reasonable to infer that, rather than an independent and casual event, HIV infection in gastric epithelial cells is tightly connected to the systemic infection status.

Inflammatory response is a characteristic feature of chronic HIV infection, which is considered to be a better indicator of AIDS progression than plasma viral load [24]. Using clinical samples, we have demonstrated that HIV infection was associated with severe inflammatory response in gastric mucosa, shown by aberrant activation and infiltration of immune cells. Further, coimmunofluorescence staining revealed phosphorylation of STAT-3 and RelA, as well as production of both TNF-α and IL-6 in both HIV-infected and bystander naive epithelial cells. Therefore, the present data suggested that HIV infection
in gastric epithelial cells plays a crucial role in HIV-induced inflammatory response in gastric mucosa.

The binding between HIV Env protein and CD4 enables HIV to enter into the target cells [25]. However, the presence of HIV genome and protein in CD4-negative cells, as shown in the present data and previous reports, suggests CD4-independent mechanisms [9, 26, 27]. It is documented that the R5 but not X4 viral strain was capable of infecting oral epithelial cells [28]. In contrast, human genital epithelial cells were found to be selectively susceptible to the X4 viral strain, and preincubation of these cells with recombinant human stromal cell-derived factor 1 protein markedly inhibited HIV infection [29]. Selective permission of distinct HIV strains suggested that coreceptors play a role in HIV infection in CD4-negative cells. Recently, accumulating studies indicate endocytosis as an alternative mechanism for HIV entry. It is shown that HIV viral particles were segregated in endosomes, and inactivation of endosomal enzymes facilitated HIV infection [30, 31]. Furthermore, inhibition of clathrin-mediated endocytosis reduced either HIV-cell fusion or infection in HeLa-derived cells [32]. In this study, we failed to observe HIV particles with endocytic vacuole in the cytoplasm of gastric epithelial cells. Therefore, we suppose that an endocytosis-independent mechanism might be involved in HIV infection in gastric epithelial cells. More work is still needed to determine the precise molecular mechanisms that enable HIV entry in gastric epithelial cells. In addition, studies examining whether gastric epithelial cells support HIV replication as well as virion package and budding would lead to better understanding of HIV infection and AIDS-associated pathology in gastric mucosa.
Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. These posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes


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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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