Role of intestinal myofibroblasts in HIV-associated intestinal collagen deposition and immune reconstitution following combination antiretroviral therapy

David M. Asmuth, Irina V. Pinchuk, Jian Wu, Gracie Vargas, Xiaoli Chen, Surinder Mann, Anthony Albanese, Zhong-Min Ma, Ramez Saroufeem, Gregory P. Melcher, Paolo Troia-Cancio, Natalie J. Torok, Christopher J. Miller and Don W. Powell

Objective: To investigate the potential role of mucosal intestinal myofibroblasts (IMFs) in HIV and associated fibrosis in gut-associated lymphoid tissue.

Design: Profibrotic changes within the secondary lymphoid organs and mucosa have been implicated in failed immune reconstitution following effective combination antiretroviral therapy (cART). Microbial translocation is believed to be sustaining these systemic inflammatory pathways. IMFs are nonprofessional antigen-presenting cells with both immunoregulatory and mesenchymal functions that are ideally positioned to respond to translocating microbial antigen.

Methods: Duodenal biopsies, obtained from patients naive to cART, underwent trichrome staining and were examined for tissue growth factor-beta (TGF-β) expression. Combined immunostaining and second harmonic generation analysis were used to determine IMF activation and collagen deposition. Confocal microscopy was performed to examine IMF activation and Toll-like receptor (TLR)4 expression. Finally, primary IMF cultures were stimulated with lipopolysaccharide to demonstrate the expression of the inflammatory biomarkers.

Results: The expression of the fibrosis-promoting molecule, TGF-β1, is significantly increased in duodenal biopsies from HIV patients naive to cART, and negatively correlated with subsequent peripheral CD4+ recovery. The increase in TGF-β1 coincided with an increase in collagen deposition in the duodenal mucosa in the tissue area adjacent to the IMFs. We also observed that IMFs expressed TLR4 and had an activated phenotype since they were positive for fibroblast activation protein. Finally, stimulation of IMFs from HIV patients with TLR4 resulted in significantly increased expression of profibrotic molecules, TGF-β1, and interleukin-6.

Conclusion: Our data support the hypothesis that activated IMFs may be among the major cells contributing to the profibrotic changes, and thus, the establishment and maintenance of systemic inflammation interfering with immune reconstitution in HIV patients.

Keywords: collagen A, gut-associated lymphoid tissue, HIV, immune reconstitution, intestinal fibrosis, intestinal myofibroblasts, lipopolysaccharide, tissue growth factor-beta, Toll-like receptor 4

University of California Davis Medical Center, Sacramento, California, University of Texas Medical Branch, Galveston, Texas, Veteran’s Administration Northern California Healthcare System, Sacramento, and California National Primate Research Center, Davis, California, USA.

Correspondence to Professor David M. Asmuth, MD, University of California, Davis Medical Center, 4150 V Street, PSSB G-500, Sacramento, CA 95617, USA.
Tel: +1 916 734 8695; fax: +1 916 734 7766; e-mail: david.asmuth@ucdmc.ucdavis.edu
*David M. Asmuth and Irina V. Pinchuk contributed equally to the writing of this article.
Received: 31 May 2014; revised: 15 February 2015; accepted: 19 February 2015.

DOI:10.1097/QAD.0000000000000636
Introduction

Effective combination antiretroviral therapy (cART) has dramatically impacted the morbidity and mortality associated with HIV infection [1–3]. Among those who achieved CD4+ T-cell counts in the normal range, HIV has become a manageable chronic disease with mortality approaching uninfected matched populations [4]. However, despite undetectable plasma HIV viral loads (pVLs), up to 20% of patients fail to realize complete immune reconstitution in the peripheral vascular compartment as measured by CD4+ T-cell count [5–7]. This subgroup of patients is at a highest risk for residual morbidity from HIV infection [8–10], with increased frequency of AIDS and non-AIDS-defining malignancies, and complications of hepatic, metabolic, and cardiovascular diseases [10–14].

Incomplete immune reconstitution is an important challenge facing patients and providers with no effective interventions available. Treatment intensification with additional or more potent cART, cytokine therapy, and therapeutic vaccination has not had a clinically meaningful impact till the present date [15–19]. Age, especially over 40–45 years old, and lower naive CD4+ T cells prior to initiating cART are the most reliable baseline characteristics associated with blunted CD4+ T-cell rise [20,21]. Since most of the HIV viral replication occurs within the lymphoid tissue, reconstitution of the T cells within this compartment would be essential for the complete recovery from the disease. Thus, an understanding of the mechanisms preventing this process would be essential for the development of successful full immune reconstitution and functional cure. Recent studies point out that development of fibrosis due to the abnormal collagen deposition in the secondary lymphoid tissue is a major factor preventing successful immune restoration even during long-term cART [22,23]. The increase in the amount of collagen within the T zone of the secondary lymphoid tissue is suggested to be a prognostic marker for poor CD4+ T-cell reconstitution and development of AIDS [24]. It has been proposed that collagen deposition in secondary lymph nodes perturbs the T cells and the fibroblastic reticular cell (FRC) network, leading to the disruption of CD4+ T-cell migration and preventing T-cell access to the cytokines and growth factors [e.g. interleukin (IL)-7, lymphotoxin] essential for their homeostasis [24,25].

Reconstitution of CD4+ T cells during cART is incomplete in the gut-associated lymphoid tissue (GALT), particularly within the mucosal lamina propria effector compartment [26–29]. Failure of GALT immune reconstitution not only contributes to the development of HIV-associated enteropathy, but might also be involved in the maintenance of the viral reservoir in the gut mucosa [26]. Further, this limited reconstitution of CD4+ T cells in GALT correlates with an early and extensive collagen deposition in the terminal ileal lamina propria and Peyer patches to a greater extent than that which occurs in the lymph nodes [27]. Although the mechanisms of HIV-associated GALT fibrosis are unknown, attention has turned to the potential consequences of sustained immune activation driven by microbial antigen translocation [30–32]. Further, fibrosis in the setting of HIV/simian immunodeficiency virus-induced chronic immune responses may be due to the unbalanced activation of the gut innate immune system toward resident gut microbiota [24,33,34]. Despite the advance in the understanding of the influence of gut microbiota to the immune reconstitution in HIV, the role of the mucosal cell network in profibrotic changes within the GALT in HIV is understudied.

We, and others demonstrated that intestinal CD90+ stromal cells (myofibroblasts and fibroblasts, IMFs) are nonprofessional antigen-presenting cells that are located beneath the epithelial basement membrane [35–38], and that play a major role in the innate gastrointestinal immune responses via signaling through Toll-like receptor (TLR)1–9 [39,40]. These cells are important contributors to the wound-healing process [41,42]. However, uncontrolled pro-inflammatory activation of IMFs leads to hardwired functional changes in these cells that may contribute to the gut fibrosis via activation of profibrogenic signaling [43]. This may result in increased collagen deposition in response to the pro-inflammatory cytokine milieu [e.g. IL-1β, IL-6, tissue growth factor-beta (TGF-β)] and also may contribute to the increase/modulation of these pro-inflammatory molecules [44,45]. Characterization of these processes has been well advanced in Crohn’s disease, in which case chronic inflammation leads to the burst of profibrogenic stimuli from professional immune and stromal cells, leading to the downstream activation of collagen deposition by the IMFs [46]. Thus, in this present study, we have extended our investigations and have analyzed the profibrotic changes in the duodenum from the HIV-positive patients, and determined whether CD90+ stromal cells are activated and thus may contribute to the profibrotic changes within the gastrointestinal mucosa in HIV.

Materials and methods

Clinical trial design and tissue processing

Treatment-naive HIV patients underwent upper endoscopy before and 9 months after starting cART. Duodenal specimens from HIV-negative volunteers were used as a control group. The negative control patients were of similar age and lifestyle as the HIV-infected patients enrolled in our GALT cohorts. All participants signed an informed consent form approved by the UC Davis Institutional Review Board prior to initiation of the study procedures. Duodenal tissue was snap-frozen for RNA

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
cells of et al. 

quantification as previously described [47].

isolated cells was analyzed by flow cytometry analysis as we previously described [35].

Primary cultures of IMFs were generated as we have previously described [35].

Primary intestinal myofibroblast cultures

Stimulation of cultured intestinal myofibroblasts with Toll-like receptor 4 agonists

Statistical analysis

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
 Results

Baseline characteristics and virologic/immunologic outcome after 9 months of antiretroviral therapy

The samples used for this pilot study were obtained during a randomized clinical trial in which HIV-positive volunteers naïve to ART underwent upper and lower endoscopy prior to and 9 months after being randomized to either a non-nucleoside reverse transcriptase inhibitor or raltegravir, both in combination with tenofovir/emtricitabine [26]. Overall, 16 HIV patients and five controls underwent upper endoscopy, and a subset underwent flexible sigmoidoscopy. This study focuses on the 14 study participants for whom the relevant samples were available for the assays reported herein at both baseline and at 9 months after cART, and negative control volunteers who underwent the same interventions. The control patients were family and friends of the HIV patients who were at a low risk for HIV infection. The demographic profiles and results from clinical responses are reported in Table 1, as well as the peripheral blood and duodenal tissue virologic and immunologic results. Importantly, the median CD4⁺ T-cell count in the HIV patients rose from a median of 328 cells/µl (235–417) at baseline to 562 cells/µl (388–651) among those who completed 9 months of treatment (n = 14). This represented a median increase of 181 cells/µl (145–295) in the peripheral blood absolute CD4⁺ T-cell counts. All patients became undetectable after 9 months by standard commercial assays. The single-copy HIV RNA and proviral DNA assay results are listed in Table 1 for peripheral blood and tissue compartments. HIV proviral DNA declined from 208 (31–1189) to 110 (14–694) in

Duodenal tissue tissue growth factor-beta expression is increased in HIV-positive patients and correlates with increases in collagen deposition

We analyzed the mRNA levels of TGF-β1 in duodenal LP and correlated it with the level of procollagen type I (α1, procollagen A) expression. TGF-β1 mRNA levels were higher in HIV patients [335.4 (187.6–621.7)] compared to controls [57.6 (2.1–134.6), P = 0.002, respectively] (Fig. 1a). Procollagen A mRNA levels were also higher in patients [462.0 (189.0–805.5),] compared to controls [92.5 (49.7–258.3), (P = 0.048) (Fig. 1b). As would be expected and as an internal control, TGF-β1 and procollagen A expression levels were positively correlated (r = 0.65, P = 0.0018) (Fig. 1c). Conversely, tissue duodenal HIV proviral DNA did not correlate with TGF-β expression (r = 0.03, P = 0.5). Neither monocyte activation as measured by sCD14 levels, nor CD8⁺ T-cell activation percentages correlated with peripheral blood or tissue HIV proviral DNA or HIV plasma viral load (both P > 0.5). Interestingly, sCD14 and CD8⁺ T-cell activation only correlated at the 9-month time-point (r = 0.63, P = 0.01), but not at baseline pretreatment, suggesting perhaps that several possible factors influence monocyte activation prior to suppression of HIV viremia, but less so once pVL is controlled by effective cART.

Profibrotic changes in duodenal tissue from HIV-positive patients negatively correlates with the reconstitution of CD4⁺ T cells

In our next step, we analyzed whether increases in the fibrosis in duodenal lamina propria correlated with the immune reconstitution of the HIV-positive patients following initiation of ART, as has been reported in lymph node parenchyma [22]. Baseline duodenal tissue

Table 1. Demographics and summary immunologic and virologic data.

<table>
<thead>
<tr>
<th></th>
<th>Normal control</th>
<th>Baseline</th>
<th>cART 9 months</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>14</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Male/female</td>
<td>5/2</td>
<td>9/5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>Caucasian/African American/Hispanic</td>
<td>3/1/1</td>
<td>6/4/4</td>
<td>–</td>
</tr>
<tr>
<td>Median age years (IQR)</td>
<td>42 (33–44)</td>
<td>37 (28–46)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Peripheral blood CD4⁺</td>
<td>328 (235–417)</td>
<td>562 (388–651)</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma viral load (copies/ml)</td>
<td>25,600 (14,770–70,613)</td>
<td>11.5 (11.3, 750)</td>
<td>&lt;0.0001</td>
<td>–</td>
</tr>
<tr>
<td>PBMC proviral DNA (copies/10⁶ cells)</td>
<td>208 (31–1189)</td>
<td>110 (14–694)</td>
<td>0.005</td>
<td>–</td>
</tr>
<tr>
<td>Duodenal tissue proviral DNA (copies/10⁶ cells)</td>
<td>37 (3.9–193.4)</td>
<td>15.4 (2.6–105.6)</td>
<td>0.32</td>
<td>–</td>
</tr>
<tr>
<td>sCD14 (µg/ml)</td>
<td>1.9 (1.7–2.4)</td>
<td>1.7 (1.2–1.8)</td>
<td>0.01</td>
<td>–</td>
</tr>
<tr>
<td>CD8⁺/CD3⁺/HLA-DR⁺ PBMCs</td>
<td>44.2% (32.2–61.4)</td>
<td>27.7% (18.8–38.5)</td>
<td>0.03</td>
<td>–</td>
</tr>
<tr>
<td>Doudenal lamina propria CD3⁺/CD4⁺ (cells/µl)</td>
<td>845 (665–1159)</td>
<td>105 (76.5–170.5)</td>
<td>0.02³</td>
<td>–</td>
</tr>
<tr>
<td>PBMC sCD14⁺ (IQR)</td>
<td>–</td>
<td>181 (145–295)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

³Median cells/µl (interquartile range).

Normal control  Baseline  cART 9 months  P value
Fig. 1. Up-regulation of biomarkers of fibrosis is increased in duodenal tissue of untreated HIV-infected patients and predicts subsequent immunologic response to cART. Duodenal tissue mRNA levels of (a) TGF-β and (b) procollagen type I are increased in HIV-infected patients compared to controls by quantitative PCR ($P = 0.002$ and $P = 0.048$, respectively). (c) As expected, TGF-β and procollagen type I expression is positively correlated ($r = 0.65$, $P = 0.0018$). Immune reconstitution (rise in absolute PBMC T-cell counts) correlates (d) negatively with baseline tissue TGF-β expression and (e) negatively with peripheral naive CD4$^+$ T-cell proportions. Baseline PBMC-naive CD4$^+$ T cells correlate with the (f) naive CD4$^+$ T cells in the duodenal tissue compartment and (g) negatively with the TGF-β expression in the duodenal tissue. cART, combination antiretroviral therapy; PBMC, peripheral blood mononuclear cell; TGF-β, tissue growth factor-beta.
TGF-β, but not procollagen A expression, negatively correlated with subsequent rise in absolute T-cell counts ($r = -0.74$, $P = 0.0024$) (Fig. 1d). Interestingly, the baseline PBMC-naive CD4$^+$ T-cell subset [median of 39.6% (22.1, 49.0)] was the only CD4$^+$ T-lymphocyte maturational subset to correlate with TGF-β expression at baseline ($r = -0.69$, $P = 0.017$) (Fig. 1e). In parallel, the only subset of CD4$^+$ T lymphocytes to correlate between the two compartments of peripheral blood and duodenal tissue was the naive CD4$^+$ T cells ($r = 0.59$, $P = 0.016$) (Fig. 1f). The absence of a correlation between the peripheral blood absolute CD4$^+$ T-cell count and the density of CD4$^+$ T cells in the duodenal tissue ($r = 0.38$, $P = 0.15$) suggests that the correlation between the two compartments relative to the naive subsets is more likely due to systemic conditions for naive T-cell maturation as has been described in the lymph nodes previously [25]. Finally, and as others have reported regarding baseline immunologic parameters that best predict subsequent CD4$^+$ T-cell response to effective ART, baseline PBMC percentage of naive CD4$^+$ T cells correlated significantly with immune reconstitution ($r = 0.71$, $P = 0.002$) (Fig. 1g) [55,56,56]. These data strongly suggest that TGF-β expression in GALT correlates with subsequent immune reconstitution in the peripheral compartment.

Collagen deposition is increased in the duodenal lamina propria during HIV infection and is associated with the increase in the activation of the intestinal myofibroblasts

Next, we examined collagen deposition within the duodenal mucosa following trichrome stain followed by histological analysis. Hematoxylin and eosin (H&E) stain was included to identify inflammation, which was not seen (Fig. 2a). As detected by trichrome staining (Fig. 2b, collagen deposition is in blue), an increase in mucosal lamina propria collagen was observed in 10 out of 20 tested HIV-positive patients compared to one out of five control patients ($P = 0.078$). Increased collagen deposition was confirmed by SHG analysis using two-photon microscopy in a subset of six out of 10 tested HIV-positive patients, but was not observed in a healthy control group (Fig. 2c). Taken together with our quantitative mRNA data analysis (Fig. 1a), these observations strongly support the conclusion that fibrotic changes occur in the gut during chronic HIV infection. No significant decrease in the collagen deposition was observed in the tissue from patients after 9 months of cART (data not shown).

Mucosal increase in collagen A deposition is concomitant with the increase in the activation of intestinal myofibroblasts

Intestinal fibrosis is classically viewed as an inflammatory process associated with the proliferation and activation of local IMFs, leading to abnormal deposition of collagen [44,57]. We observed that the increase in deposition of collagen A in the duodenal lamina propria of HIV-positive patients (Fig. 3, in blue) was accompanied with the disruption of normal mucosal fibroblastic network architecture and activation of IMFs, as determined by expression of α-SMA (Fig. 3, in green) and fibroblasts activation protein (FAP) (Fig. 3, in red). FAP is believed to be increased in fibroblasts in fibrotic tissue and cancer [58]. Thus, colocalization of the α-SMA (Fig. 3) and the FAP (formation of the yellow-orange color on merged images, Fig. 3) within IMFs from HIV-positive patients suggests that abnormal activation of the stromal cells in the intestinal mucosa may contribute to the fibrotic tissue remodeling in HIV-associated immunopathogenesis.

Stimulation of Toll-like receptor 4 on intestinal CD90$^+$ fibroblast/myofibroblasts derived from HIV-positive patients up-regulates the production of profibrotic mediators

The main driver of gut fibrogenesis is believed to be chronic inflammation, which leads to mesenchymal cell recruitment and activation. Recent findings suggest that microbial overgrowth and abnormality in microbial translocation play a critical role in gut fibrogenesis [34,57]. LPS is known to be a profibrotic agent [59]. Thus, we determined whether IMFs express TLR4, a receptor for LPS, in duodenal mucosa from HIV-positive patients. Using immunostaining followed by confocal microscopy of duodenal mucosa tissue sections, we were able to detect compartmentalized TLR4 expression in duodenal mucosa from HIV-positive patients (Fig. 4, showing in red). In duodenal LP, CD90$^+$ stromal cells (also known as IMFs) were among the major cell phenotypes expressing TLR4 (Fig. 4, showing in green). Next, we determined how TLR4 stimulation modulates expression of the cytokine and growth factors involved in the fibrosis. Our data demonstrated that stimulation of the HIV-derived IMF primary cultures with LPS (1 μg/ml) for 24 h resulted in significant up-regulation of TGF-β1 and IL-6 mRNA expression (Fig. 5). Taken together, these data suggest that during HIV enteropathy, the interaction of IMFs with LPS may contribute to the increase in the soluble mediators of fibrogenesis (IL-6, TGF-β1) and that may directly enhance collagen deposition by IMFs.

Discussion

Incomplete immune reconstitution following effective cART is associated with significant residual morbidity amongst HIV-infected patients. Its understanding and control is a significant unmet need in the management of HIV disease. Considerable debate exists in the literature regarding the underlying pathophysiology of failed immune reconstitution in the gut following effective cART. In this study, we sought to examine the role of events at the interface of the gut mucosa and the systemic immune system to explore which factors influence peripheral CD4$^+$ T-cell reconstitution following effective
We and others have previously demonstrated that higher percentages of pro-inflammatory bacterial taxa, including the Proteobacteria/Enterobacteriales, were associated with changes in the duodenal GALT T-cell subset proportions and T-cell activation [33,60]. Similarly, Dillon et al. [61] also observed a relationship between altered mucosal microbiome and blood T-cell activation. More recently, we reported the impact of oral serum-derived bovine immunoglobulin (which is broadly reactive against bacterial and viral antigen) on HIV enteropathy, gut mucosal repair and function, and systemic immune activation [62]. Most relevant to the work described herein, oral bovine immunoglobulin administration resulted in a significant increase in duodenal lamina propria CD4⁺ T cells after only 8 weeks of treatment, supporting the hypothesis that bacterial products impact immune reconstitution locally [62]. Klatt et al. [63] observed similar results with the administration of probiotics/prebiotics in combination with ART in SIV-infected pigtail macaques, further supporting the hypothesis that microbial translocation is causing fibrosis.

The present study extends that work by exploring pathways of focal tissue inflammation by examining TGF-β expression as an effector molecule and procollagen type I expression as the downstream mediator of fibrosis.
fibrosis in duodenal tissue. We observed increased extracellular matrix deposition of collagen as detected by histopathologic measures using trichrome staining in a majority of duodenal samples and dual photon microscopy. Similar observations were previously reported by the Estes group for ileal lamina propria and Peyer patches [27]. Increased expression/production of procollagen and TGF-β1 in mucosal and lymph node tissue has been reported previously in the setting of both HIV-1 and macaque SIV infection in the cervix, lymph nodes, and GALT [64–68], demonstrating the systemic nature of HIV-associated mucosal inflammation. We were also able to demonstrate the impact of baseline profibrotic pathways on subsequent peripheral blood CD4+ T-cell immune reconstitution. Our results, which focus on events at the GALT–gut lumen interface, identify a portal for systemic inflammation and by extension, immune activation in patients on effective cART.

Although previous studies have identified lymphoid T-regulatory and double-negative lymphocytes as a potential source for the TGF-β [25,66], we sought to pursue the potential role of widely distributed IMFs in the genesis of collagen deposition in GALT of HIV patients. This cell type is of particular interest in this regard as it is of the same lineage as the stellate cell in the liver and is known to play a key role in the fibrogenesis of other inflammatory bowel diseases such as Crohn’s disease [46]. These stromal cells represent nonprofessional antigen-presenting cells which form a continuous interconnecting network under the epithelial layer of the entire gastrointestinal tract. Thus, they are ideally positioned to respond to microbial antigen translocation. When IMFs are stimulated with bacterial antigen, they increase in number and volume as demonstrated by α-SMA staining [69]. Indeed, we observed increased α-SMA staining in the duodenal mucosa of HIV-positive duodenal mucosa is adjacent to the α-SMA+ IMFs (in green), which also express marker of activation, FAP, and result in formation of orange-yellow color on merged images. FAP, fibroblasts activation protein; IMF, intestinal myofibroblast.

**Fig. 3. Intestinal myofibroblasts are source of collagen in HIV disease.** Anti-α-SMA+ mAbs (smooth muscle actin monoclonal antibodies) (clone 1A4) were used to detect myofibroblasts (shown in green), rabbit polyclonal fluorochrome conjugated anti-FAP Abs were used to analyze activation of IMFs (shown in red), collagen A deposition was detected by second harmonic generation (SHG) analysis (shown in blue) using two-photon microscopy. Increase in a ‘filament’ type fully formed collagen A in HIV-positive duodenal mucosa is adjacent to the α-SMA+ IMFs (in green), which also express marker of activation, FAP, and result in formation of orange-yellow color on merged images. FAP, fibroblasts activation protein; IMF, intestinal myofibroblast.
IMFs in the tissue samples from HIV patients. It is known from previous studies that there are increased plasma levels of LPS in HIV patients [30]. Although limited, the TLR4 staining was strongest on IMFs which are located in the subepithelial position – a location ideal for signaling microbial antigen translocation. Several pathogen-associated molecular patterns (PAMPs) recognition receptors have been detected previously on IMFs, and TLR2, 5, and 9 [45]. In the final set of experiments, we cultured IMFs from intestinal biopsy specimens and stimulated them with TLR4 agonists in order to assess whether TGF-β1 and IL-6 up-regulation could be detected. IL-6 is an important cytokine in the inflammation cascade and contributes to collagen deposition. It has been suggested to be a useful predictive marker for liver fibrosis in HIV-infected patients with alcohol addiction [70]. Consistent with animal models and other disease state reports [71,72], the IMFs had a significant increased expression of TGFβ1 and IL-6, further supporting the proposed hypothesis. These data suggest that a complex of profibrotic molecules may be released by IMFs upon microbial stimulation in the HIV leaky gut which will require further investigation to verify.

A strong negative correlation was observed between baseline duodenal TGF-β expression and systemic immune reconstitution following 9 months of effective cART. A similar correlation was observed between baseline duodenal TGF-β expression and systemic naive CD4+ T-cell percentages. Zeng et al. [25] have presented an important set of experiments demonstrating the impact of lymph node fibrosis and loss of normal architecture in the FRc network on IL-7 signaling which is necessary for naive CD4+ T-cell survival. The findings
Intestinal myofibroblasts have been shown to impact T-regulatory cell activation and function [51], and are anatomically ideally situated to respond to microbial antigen translocation in the setting of HIV disease. Perhaps, these cells are what link failure of immune reconstitution despite virologic control. Although these present data do not prove, nor do they attempt to prove, a causal relationship between IMFs and up-regulated systemic profibrotic pathways, understanding the role of IMFs at the front gate of microbial antigen–mucosal immunity interactions may provide a rational strategy for restoring microbial communities and interrupting focal/ systemic profibrogenic pathways. These novel findings in the small intestines of HIV patients may provide insights into alternative therapeutic opportunities to improve immune reconstitution and reduce associated morbidity.

Acknowledgements

The research was made possible by grant number UL1 RR024146 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health, and NIH Roadmap for Medical Research NIH grant support NIDDK (IR1DK103150-01A1), NCATS (KL2TR000072), NCATS (TR000071), and the Investigator-Initiated Studies Program of Merck & Co., Inc.

We are especially grateful to the nurses on the clinical research unit of the UCD CCCR and the patients for their commitment to research efforts. We would also like to acknowledge and thank Dr Taewook Chun of the NIH who performed the HIV RNA and proviral DNA assays.

Conflicts of interest

The opinions expressed in this study are those of the authors and do not necessarily represent those of Merck & Co., Inc. The contents do not necessarily represent the views of the Department of Veterans Affairs or the United States Government.

References


Intestinal myofibroblasts in HIV-associated GALT fibrosis Asmuth et al.


