Antigen-Presenting Cell Candidates for HIV-1 Transmission in Human Distal Colonic Mucosa Defined by CD207 Dendritic Cells and CD209 Macrophages

Gloria C. Preza,¹ Karen Tanner,² Julie Elliott,² Otto O. Yang,²⁻⁴ Peter A. Anton,² and Maria-Teresa Ochoa¹

Abstract

A common route for HIV-1 infection is sexual transmission across colorectal mucosa, which is thought to be 10–2,000 times more vulnerable to infection than that of the female genital tract. Mucosal surfaces are the first line of defense against many pathogens but the antigen-presenting cells (APCs), key regulators of innate immunity and determinants of adaptive immunity, are not well defined in these target tissues. Using immunohistochemistry, dendritic cells expressing Langerin (CD207⁺), a lectin known to bind and internalize HIV-1, were detected in the periphery of colonic glands and sparsely scattered in the submucosa similarly in colorectal mucosa. This cell type, well known in skin, has generally not been reported in colonic/rectal mucosa. Unexpectedly, the largest APC population observed was a macrophage-like population expressing the well-characterized tissue macrophage markers CD68 and CD163. Confocal microscopy of these cells revealed colocalization of CD209 (DC-SIGN), a presumed dendritic cell marker believed to facilitate HIV-1 transmission, but not other dendritic cell markers. These results show evidence of the unconfirmed presence of Langerhans cells in colorectal mucosa and a predominance of macrophage-like APCs that express CD209 (DC-SIGN). These findings define potential target cells in the pathogenesis of HIV-1 transmission, which may have key implications for the study of early transmission events in normal colorectal mucosa, as well as other infectious diseases and primary immune diseases involving the gut.

Introduction

MUCOSAL INNATE IMMUNE CELLS comprise a diverse group of cells that plays important roles in the induction and regulation of immune responses at mucosal surfaces. The most important innate immune cells are the professional antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages. A key DC function is antigen presentation to T lymphocytes, whereas macrophages are more important for direct effector functions such as microbe recognition and killing.¹⁻³ In peripheral tissues, these cell types are among the first to sense invading pathogens through pattern recognition receptors (PRRs) that recognize conserved molecular motifs of microbes. DC and macrophage functions vary in different tissues due to differences in expression of PRRs and antigen exposure,² which

in turn direct the induction of local and adaptive immune responses, both of which are thought critical to successful prevention of HIV mucosal infection.

APCs in the skin, mouth, small intestine, and the female genitourinary tract have been well defined.^{1,2,4-7} However, thorough characterization in the mucosa of the distal colon and rectum mucosa, the most vulnerable sexually exposed mucosa, is lacking. To date, APCs in the lower gut mucosa are poorly understood and are primarily derived from studies of inflammatory bowel disease (IBD) and animal models.⁶ Given that the sigmoid colon and rectal mucosae are an epidemiologically significant portal of entry for sexually transmitted pathogens, identifying the population of APCs in these anatomical regions is vital to developing prevention strategies for various pathogens, including HIV and other sexually transmitted infections.

¹Department of Dermatology, Keck School of Medicine, University of Southern California, Los Angeles, California.

²Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, California.

³Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles, California.

⁴AIDS Healthcare Foundation, Los Angeles, California.

Rectal mucosa is the primary route of HIV-1 infection in men who have sex with men and an underappreciated (and possibly predominant) contributor to heterosexual infections. This tissue is exquisitely vulnerable to transmission, with 10- to 2,000-fold relative risk per sexual act compared to vaginal-cervical exposure, which is further increased when coexisting infections are present.^{8–11} Moreover, rectal mucosal tissue constitutes a key anatomical reservoir for established HIV-1 infection,^{12,13} is the first compartment for CD4⁺ T cell depletion in new infections (regardless of the route of exposure), and is rich in virus-permissive immune cells, including DCs, macrophages, and activated CD4⁺ T cells.^{14,15}

Within mucosal tissues, DCs may be among the first cells to encounter HIV-1 and have been hypothesized to play a crucial role in initial virus dissemination by transporting HIV-1 to CD4⁺ T lymphocytes in regional lymph nodes.^{14,16,17} Moreover, DCs and monocyte/macrophages are a key determinant of the activation state of CD4⁺ T lymphocytes in mucosal tissues. Thus mucosal innate immune function has critical implications for infectious diseases as well as autoimmunity and even systemic immune regulation. Therefore, detailed evaluation of APCs of this tissue site in normal subjects is important to establish baseline populations for comparison with altered states. This article describes a detailed evaluation of APCs using frozen tissue from two regions of this important, HIV-exposed tissue compartment (rectum and sigmoid colon) in normal subjects, using immunohistochemistry (IHC) and confocal microscopy.

Materials and Methods

Human normal sigmoid colon and rectal mucosal tissue

Gastrointestinal mucosal biopsies were freshly acquired from the sigmoid colon and rectum as previously described.¹⁸ Briefly, healthy HIV-seronegative participants without evidence of rectal sexually transmitted disease (confirmed) or chronic gastrointestinal disorders were recruited to undergo flexible sigmoidoscopy. The study was approved both by the USC and UCLA Offices of the Human Research Protection Program Institutional Review Board and all subjects provided written informed consent. Using large-cup endoscopic biopsy forceps (Microvasive Radial Jaw #1589, outside diameter 3.3 mm), two to three biopsies (approximately 1 mm³) were acquired endoscopically first from a location 5–8 cm from the anal verge (“rectal”) and then another two or three biopsies from 25–30 cm (“sigmoid”). In the endoscopy suite, biopsy specimens were immediately embedded in OCT medium (Ames Co., Elkhart, IN) and snap-frozen in liquid nitrogen.

Immunohistochemistry of dendritic cells, macrophages/monocytes in normal sigmoid colon, and rectal mucosa

Cryostat sections (4 μ m each) were acetone fixed and blocked with normal horse serum and then sequentially incubated with the indicated murine antihuman antibodies, biotinylated horse antimouse IgG and an avidin/biotin-peroxidase. Tissues were counterstained with hematoxylin (VWR, Radnor, PA) for light microscopy (Olympus BX51 microscope, Central Valley, PA, equipped with an Olympus

America camera). The following mouse antihuman antibodies were used: CD1a (clone NA1/34-HLK; AbD Serotec, Raleigh, NC), CD1b (clone SN13 K5-1B8; eBiosciences, San Diego, CA), CD1c (clone M241; Ancell, Bayport, MN), CD207 (clone DCGM4; Immunotech SAS, Beckman Coulter, Marseille, France), CD14 (clone RPA-MI; Zymed Laboratories, South San Francisco, CA), CD68 (clone EBM11; DAKO, Carpinteria, CA), CD163 (clone EDHu-1; AbD Serotec, Raleigh, NC), and CD209 (clone DCN46; BD Pharmingen, San Diego, CA).

Enumeration of immune cells

To identify and quantify immune cells in sigmoid and rectal tissue, immunostained cells in epithelium and lamina propria were enumerated and reported per mm² for each area. Slides were scanned using an Aperio ScanScope XT (Aperio Technologies, Inc., Vista, CA) and images were analyzed using ImageScope Aperio software. On each slide, three separate, nonoverlapping areas were circumscribed for counting; each area encompassed 10 colonic/rectal glands; cells were counted at 40 $\times$. Cells were categorized as positive if a nucleus was surrounded by brown staining for the particular cell marker in all three areas for each tissue section and normalized to cells/mm². For statistical analyses, averages of total cell counts (per mm²) in three areas were sampled (Student's *t*-test).

Two-color immunofluorescence staining of frozen cryostat sections

Indirect immunofluorescence was performed by serially incubating cryostat-prepared tissue sections with mouse antihuman monoclonal antibodies (mAbs) to CD1a, CD207, CD209, CD68, and CD163 of different isotypes followed by incubation with isotype-specific fluorochrome- (Alexa 488 and Alexa 568, Molecular Probes, Eugene, OR) labeled goat antimouse Ig antibodies. Controls included were isotype-matched control mouse antibodies as well as specific mouse antibodies with a mismatched secondary antibody. Slides were mounted with ProLong Gold antifade reagent with a nuclear marker DAPI (Molecular Probes, Eugene, OR). Images were obtained using confocal laser microscopy.

Confocal microscopy

Frozen cryostat sections were examined using a Leica-TCS-SP5 MP inverted confocal laser-scanning and two-photon laser microscope (Heidelberg, Germany) fitted with DPSS-diode (561 nm), argon (488 nm), and two photon laser (Spectra-Physics Millennia X 532 diode pump laser and Tsunami picosecond Ti-sapphire laser) at the Craniofacial Center for Molecular Biology at USC. Sections were illuminated with 488/568 nm for green/red and DAPI for nuclear label. Images of specimens with Alexa 488, Alexa 568 were recorded sequentially through the spectral emission filters set from 500 to 550 nm for Alexa 488, 580 to 700 nm for Alexa 568, and 410 to 490 nm for DAPI. Pairs of single images were superimposed for colocalization analysis. All micrographs were compiled in serial Z-stack images 0.1–1.0 μ m apart. All images were analyzed and normalized using LAS AF Lite software, Leica Microsystems (Heidelberg, Germany). Percentage double positive cells was assessed in three high-power fields in at least three sections.

FIG. 1. Identification of Langerin⁺ dendritic cells (DCs) in normal human sigmoid colon and rectum. Normal human sigmoid colon (**A**) and rectum (**B**) were labeled with monoclonal antibodies (mAbs) for CD207 (Langerin) and CD1a and visualized using the immunoperoxidase method. CD207 and CD1a were mostly located surrounding colon/rectal glands intraepithelial in between goblet cells and in the basement membrane. Inset: Langerhans cell displaying characteristic dendritic morphology. Original magnification 400 $\times$. Bar represents 100 μ m. Images are representative of five different subjects.

Results

Identification and characterization of DCs expressing CD207 and CD1 in normal human sigmoid colonic and rectal mucosa

Cells with dendritic morphology were detected in both mucosal tissue sites in all subject's samples (Fig. 1). These DCs were predominantly located intraepithelially (between goblet cells). The dendritic morphology and presence of these characteristic markers (CD1a/CD207) suggest these could be Langerhans cells (LCs). Cells expressing other DC markers such as CD1b and CD1c were found in the periphery of colonic/rectal glands (Fig. 2). By contrast, evaluation for markers of mature DCs such as CD83 and CD208 (DC-LAMP) identified very few cells expressing these markers in either sigmoid colon or rectum as previously reported in the skin.

Distribution and morphology of cells expressing macrophage markers in human sigmoid colon and rectum

Cells expressing the characteristic monocyte/macrophage markers CD14, CD68, and CD163 were distributed from the lamina propria to the muscularis mucosae and were similarly located in both the sigmoid colon and rectum in all subject's samples (Fig. 3). These macrophage-like cells were distinct from DCs (cells expressing CD207 and CD1) and were found in different areas (IHC and CF) (Fig. 1). However, these macrophage-like cells did appear to have a distribution

FIG. 2. Identification of CD1 dendritic cell markers in normal human sigmoid colon and rectum. Normal human sigmoid colon and rectum were labeled with mAbs for CD1b and CD1c and visualized using the immunoperoxidase method. CD1b and CD1c were located below simple columnar epithelium and interdispersed around colon/rectal glands in the lamina propria. Original magnification 400 $\times$. Bar represents 100 μ m. Images are representative of five different subjects.

FIG. 3. Morphology and distribution of monocyte/macrophage cell markers in normal human sigmoid colon and rectum. Normal human sigmoid colon and rectum were labeled with mAbs for CD14, CD68, CD163, and CD209, and were visualized using the immunoperoxidase method. CD14, CD68, CD163, and CD209 were all found below simple columnar epithelium, mostly expressed throughout the lamina propria down to the muscularis mucosae layer, and in the lamina propria separating colon/rectal glands (A,C). Images were taken from both tissues for each marker. The distribution was determined with an original magnification of $\times 200$ (A,C) and the cell morphology is shown with a magnification of $\times 400$ (B,D). Monocyte/macrophage cells display dendritic cell morphology (insets). Bar = $50 \mu\text{m}$. Pictures are representative of five different subjects.

similar to DC-SIGN (CD209)-expressing DCs (Fig. 3). The morphologies of these cells ranged from long and spindle to round and stellate (Fig. 3, inset).

Macrophages are more abundant than DCs in human sigmoid colon and rectum

In situ imaging analysis was used to enumerate cells expressing DC or monocyte/macrophage markers in freshly acquired colonic and rectal biopsies from five healthy volunteers without known gastrointestinal pathology. Ten colonic and 10 rectal glands were scanned per person (including epithelium and lamina propria above the muscularis mucosae, excluding

lymphoid aggregates). Of all the characterized APCs quantified here, there were significantly greater numbers of macrophage-like cells (expressing CD14, CD163, CD68, and CD209) ($875\text{--}1,487 \text{ cells/mm}^2$) in both rectum and sigmoid colon locations than dendritic cells expressing CD1 and CD207 ($84\text{--}188 \text{ cells/mm}^2$), a consistent finding in all subjects ($p < 0.001$; Mann-Whitney rank sum test) (Fig. 4 and Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/aid). The numbers of cells within each APC type were similar regardless of whether they were from the sigmoid colon or rectum. However, there were statistically significant differences between the number of DC cell subsets and monocyte/macrophage cell subsets at both sites ($p < 0.001$),

FIG. 4. DC and monocyte/macrophage enumeration in normal human sigmoid colon and rectum. Quantitative enumeration was performed at the single cell level in immunohistochemically stained sections by *in situ* analysis using aperio imaging software. $N=5$. Maximum, minimum, and median values $\pm$ SEM are shown. * $p=0.023$, ** $p=0.007$, n.s. = not significant.

Mann-Whitney rank sum test. There were no statistically significant differences between the two anatomical sites for most other markers, with the exception of CD1b (rectum > sigmoid: $p=0.023$) and CD14 (sigmoid > rectum; $p=0.007$; by Student's *t*-test) (Fig. 4). Thus the major population of innate immune cells in both the rectum and the sigmoid colon consists of macrophage-type cells expressing CD163, CD14, CD68, and CD209 that are present in nearly a 7-fold excess compared to DCs.

CD209 (DC-sign) is expressed on different cell subsets than DCs, and colocalizes with the macrophage cell markers CD68 and CD163, in human sigmoid colon and rectum

Based on the similar distribution of CD209 (DC-SIGN) and the macrophage markers CD68, CD163, and CD14, as determined by immunohistochemistry, we decided to examine the phenotype of CD209⁺ cells in human sigmoid colon and rectum. Double immunofluorescence labeling of frozen tissue sections with antibodies to CD209 was performed and paired with DC markers (CD1a, CD1b, CD1c, and CD207) and macrophage markers (CD68 and CD163). Confocal microscopy analysis revealed that CD209⁺ cells did not colocalize with conventional DC markers CD1a, CD1b, CD1c, and CD207 (Fig. 5). DC-SIGN expression (CD209)

FIG. 5. CD209⁺ (DC-SIGN) cells are expressed on different cells subsets than DC markers. Normal human rectum was labeled with mAbs for CD209, CD207, CD1a, CD1b, and CD1c and then visualized using confocal microscopy. CD209⁺ (red images) did not colocalize with CD207 or CD1a (green images), markers for Langerhans cells. CD209 did not colocalize with the CD1 markers CD1b and CD1c. Nuclei were labeled with DAPI (blue images). Inset corresponds to a zoomed area marked by white arrows. Original magnification $\times 630$, bars = 50 μ m. Lu, lumen; GL, gland.

FIG. 6. CD209⁺ (DC-sign) cells and CD68⁺, CD163⁺ monocyte/macrophage cell markers colocalize on the same cell subsets in normal sigmoid colon and rectum. Normal human sigmoid colon and rectum were labeled with mAbs for CD209, CD68, and CD163 and visualized using confocal microscopy. CD209 (red images) colocalized with CD68 and CD163 (green images) monocyte/macrophage cell markers. Nuclei were labeled with DAPI (blue images). Inset corresponds to magnified positive cells marked by white arrows. Original magnification $\times 630$, bars = 50 μm . Lu, lumen; GL, gland.

was predominantly found coexpressed on macrophage-type cells (CD68⁺/CD163⁺) and not on DC-type cells (CD1a,b,c⁺/207⁺) and were located periapically near the base of the colonic glands (Fig. 6). Not all CD209⁺ cells were double positive. We find that approximately 70.3% of CD209⁺ immune cells were double positive for CD209/CD68 and CD209/CD163.

Discussion

We demonstrate that the APC population in normal human sigmoid colon and rectum is composed of two phenotypic subpopulations. The first are immature dendritic cells that express CD1 and CD207, and the second are macrophages/monocytes that express CD14, CD68, CD163, and CD209 (Fig. 7). We conclude that CD209⁺/CD68⁺/CD14⁺/CD163⁺ cells are the most abundant innate immune cells at the colorectal mucosa, and may be an important component of the innate immune response at the mucosa.

Given the proposed role of CD207 (Langerin) in HIV-1 transmission¹⁶ and the key role of CD207⁺ and CD1a-expressing LCs in skin and various mucosal surfaces, we in-

vestigated the *in situ* distribution of CD207 and CD1a in normal human sigmoid colon and rectum (Supplementary Fig. S1). To our knowledge LCs, a myeloid DC subtype common in stratified epithelium,^{19,20} have not been thoroughly examined in these microcompartments. LCs can be defined by the expression of the PRR Langerin,²¹ a C-type lectin and CD1a, that presents lipid and glycolipid antigens to T lymphocytes.^{22–25} LCs have been described in the skin, mucosa of the mouth, foreskin, and vagina,²⁶ but there is a paucity of direct evidence showing expression of LCs in rectal epithelial tissue.¹⁶ We identify cells with typical dendritic cell morphology expressing CD1a and CD207 predominantly intraepithelially and surrounding the colonic/rectal glands but sparse in the lamina propria (LP) where they are seen directly below the epithelium (Fig. 1). There are about 84–188 cells/mm² (Fig. 4 and Supplementary Table S1), similar to the densities of LCs observed in human foreskin,²⁷ human penile explant tissue,^{20,28} and vagina,²⁶ but far less than is seen in the epidermis of the skin.²⁹ Although sparse, the intraepithelial location of these CD1a⁺ and CD207⁺ cells is consistent with the notion that in some areas of the small intestine of mice dendritic cells form intraepithelial dendrites that extend into the

FIG. 7. DC and macrophage subsets as HIV targets in distal colonic and rectal mucosa. Antigen-presenting cells consists of at least two populations: a small population that expresses CD207 and CD1 and a major population of CD14, CD68, CD163, and CD209 positive cells. CD209 (DC-SIGN) and CD207 (Langerin) are pathogen recognition receptors important in HIV infection and transmission.

lumen for antigen sampling.¹ Further analysis of these CD1⁺ and CD207⁺ cells in the human colon and rectum could provide an indication of their function in relation to this specific location in the human colorectal mucosa. Langerin has been reported in cells that differ from LCs in mice^{30–32} and in Langerin⁺ monocyte-derived DCs from humans.^{33,34} Although we believe these colorectal CD1⁺ and CD207⁺ cells to be LCs, it cannot be ruled out that the Langerin⁺ cells expressed in the colorectal mucosa may differ from those found in the epidermis of other tissues.

The ability of DCs to process and present different antigens to T cells relies in part on the expression of the CD1 family glycoprotein receptors. Apart from CD1a, CD1b and CD1c are also capable of presenting various classes of lipid antigens and are involved in cross presentation of antigen, which is thought to boost CD8⁺ T lymphocyte responses.^{35,36} We find that these molecules have a similar distribution to CD1 and are mainly localized intraepithelially and within the lining of the basement membrane (Fig. 2). Previous studies describe DCs as the most abundant population in the human colonic lamina propria. DCs were found to be the major APC in the colonic and rectal isolated lymphoid follicles in humans and these HLA⁺DR⁺ DCs form a reticular framework throughout the lamina propria and beneath the basement membrane of the colonic crypts.¹ However, those studies analyzed surgically resected colonic mucosa from patients with cancer and other nonmalignant conditions.^{37,38} Previous studies investi-

gating the reactivity of lamina propria T cells to commensal bacteria determined, using flow cytometry, that the majority of DCs isolated from the lamina propria of human small intestine and colon were CD1c⁺ DCs.^{39,40} We show that freshly acquired, endoscopic biopsy tissues from healthy sigmoid colon and rectum have what appear to be LCs located in the epithelium and a second group of DCs comprised of either CD1a⁺/CD1b⁺ and CD1c⁺ cells inside and around the glands.

Another key C-type lectin found on DCs besides Langerin (CD207) is DC-SIGN (CD209), which is expressed on non-Langerhans DCs in skin subepithelium. Our results show that a large population of cells express CD209, are directly below the mucosal epithelium, and are distributed throughout the lamina propria, between colon/rectal glands, extending to the muscularis mucosae (Fig. 3). However, consistent with prior reports that this molecule can be expressed on macrophages⁴¹ we find that CD209 is coexpressed with the monocyte/macrophage markers CD14 (coreceptor for lipopolysaccharide), CD68 (tissue macrophage surface molecule that binds LDL), and CD163 (haptoglobin receptor involved in the transport of iron into cells), but not with DC markers CD1a, CD1b, CD1c, and CD207 as previously described in skin and the renal interstitium.⁴² Krutzik *et al.* demonstrated that activation of Toll-like receptors causes rapid differentiation of distinct precursors of human peripheral blood monocytes into CD1b and CD209 (DC-SIGN) cells.⁴³ Montoya *et al.* further showed the ability of distinct innate immune cytokines to trigger macrophage functional programs such as phagocytosis, and the ability of interleukin (IL)-15 to induce the coexpression of CD209 and CD163 on macrophages and not CD1b (dendritic cell-specific marker).⁴⁴ These studies provide evidence that the dual host defense roles of the innate immune system are mediated by induction of two distinct phenotypic and functional populations.

DCs and macrophages are candidate cells for early transmission events in HIV pathogenesis and it is unclear which cell type(s) HIV-1 must reach to establish systemic infection. Knowing which specific DC and macrophage cell subtypes are present at the colorectal mucosa may have implications for the pathogenesis of HIV-1 transmission, for which the early events involving rectal transmission are poorly understood. Macrophages are susceptible to HIV-1 infection and are productively infected during chronic infection *in vivo*.^{28,45} It has been suggested that these cells may contribute to HIV-1 entry in penile urethral and vaginal mucosal sites.²⁸ Furthermore, the cell surface molecule DC-SIGN expressed on DCs has been proposed to be important for binding and transporting HIV-1 from mucosa to regional lymph nodes,^{14,16,17} although we find this molecule on macrophages and not DCs.

We speculate that the high density of mucosal macrophages (and not DCs) expressing CD209 may possibly contribute to the higher efficiency of rectal transmission, but additional studies are warranted in support of this notion. Although the DCs in sigmoid colonic and rectal mucosa do not express CD209, they express Langerin and may appear to be LCs, a myeloid DC subtype common in stratified epithelium^{19,20} that is capable of uptake and degradation of HIV-1 in Birbeck granules^{16,46} or facilitation of its spread.⁴⁷ It is thus unclear whether LCs might play a facilitating or inhibiting role in HIV-1 transmission rectally. Even though this study did not examine plasmacytoid DCs, it has been shown that

plasmacytoid DCs, which play an important role in HIV-1 immunopathogenesis, are recruited into mucosal surfaces including the colon during SIV infection.^{48,49}

In agreement with our results McElrath *et al.* very recently reported the presence of CD68⁺ macrophages expressing CCR5 more in the rectum compared to colon, using fluorescence microscopy and superimposed images to establish expression of both markers on the same cell.⁵⁰ Using confocal microscopy we better define a CD209⁺/CD163⁺/CD68⁺ macrophage population in human colon and rectum that does not express characteristic DC markers CD1a, CD1b, CD1c, and CD207.

In summary, we characterized the distribution and phenotype of colorectal APCs, important innate immune cells for regulating immune responses at mucosal surfaces. We examined the expression in sigmoid colon and rectum of key pathogen recognition receptors involved in innate immune regulation and HIV-1 pathogenesis, finding that the dominant APCs are rectal macrophages expressing DC-SIGN, which are strategically positioned adjacent to the epithelium primed to interact with microorganisms and microbial products that have breached the epithelium. Identification and understanding of the resident mucosal innate immune system and the knowledge of specific cell candidates at the colonic/rectal mucosa are critical to informing rectal microbicide drug development, HIV-1 vaccine design, and the pathogenesis of other mucosa-associated infections and inflammatory bowel disease.

Acknowledgments

We thank Chris Creencia of the UCLA Tissue Procurement Laboratory Core (TPLC) facility for cutting sections for immunohistochemistry according to our specific SOP, the USC Tissue and Cell Imaging Core facility for scanning slides using the Aperio ScanScope, and the Center for Craniofacial Molecular Biology at the USC Ostrow School of Dentistry for the use of the Leica SP5 confocal microscope. Deep appreciation is owed to Dr. Wilfred Wienstein, UCLA, for having instilled an appreciation for the accuracy of the directly visible.

Support from the UCLA CFAR Mucosal Immunology Core (AI28697) is acknowledged.

Other support includes a Ford Foundation Postdoctoral Fellowship (G.C.P.) and NIH R56 AI100638 (O.O.Y.).

Author Disclosure Statement

No competing financial interests exist.

References

- Iwasaki A: Mucosal dendritic cells. *Annu Rev Immunol* 2007;25:381–418.
- Kelsall B: Recent progress in understanding the phenotype and function of intestinal dendritic cells and macrophages. *Mucosal Immunol* 2008;1:460–469.
- Modlin RL: Innate Immunity: Ignored for decades, but not forgotten. *J Invest Dermatol* 2011;132:882–886.
- Ochoa MT, Loncaric A, Krutzyk SR, Becker TC, and Modlin RL: 'Dermal dendritic cells' comprise two distinct populations: CD1⁺ dendritic cells and CD209⁺ macrophages. *J Invest Dermatol* 2008;128:2225–2231.
- Iijima N, Thompson JM, and Iwasaki A: Dendritic cells and macrophages in the genitourinary tract. *Mucosal Immunol* 2008;1:451–459.
- Fries PN and Griebel PJ: Mucosal dendritic cell diversity in the gastrointestinal tract. *Cell Tissue Res* 2010;343:33–41.
- Mascarell L, *et al.*: Oral dendritic cells mediate antigen-specific tolerance by stimulating TH1 and regulatory CD4⁺ T cells. *J Allergy Clin Immunol* 2008;122:603–609.
- Vittinghoff E, *et al.*: Per-contact risk of human immunodeficiency virus transmission between male sexual partners. *Am J Epidemiol* 1999;150:306–311.
- Gray RH, *et al.*: Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *The Lancet* 2001;357:1149–1153.
- Sartori E, *et al.*: Herpes simplex virus type 2 infection increases human immunodeficiency virus type 1 entry into human primary macrophages. *Virol J* 2011;8:166.
- Keller MJ and Herold BC: Impact of microbicides and sexually transmitted infections on mucosal immunity in the female genital tract. *Am J Reprod Immunol* 2006;6:56–63.
- Di Stefano M, *et al.*: In vitro productive infection of non polarised cervical and rectal biopsies by syncytium-inducing and non syncytium inducing primary HIV-1 isolates. *New Microbiol* 2004;7:1–74.
- Poles MA, *et al.*: Lack of decay of HIV-1 in gut-associated lymphoid tissue reservoirs in maximally suppressed individuals. *J Acquir Immune Defic Syndr* 2006;43:65–68.
- Gurney KB, *et al.*: Binding and transfer of human immunodeficiency virus by DC-SIGN⁺ cells in human rectal mucosa. *J Virol* 2005;79:5762–5773.
- Anton PA, *et al.*: Enhanced levels of functional HIV-1 co-receptors on human mucosal T cells demonstrated using intestinal biopsy tissue. *AIDS* 2000;14:1761–1765.
- De Witte L, Nabatov A, and Geijtenbeek TBH: Distinct roles for DC-SIGN⁺-dendritic cells and Langerhans cells in HIV-1 transmission. *Trends Mol Med* 2008;14:12–19.
- Mesman AW and Geijtenbeek TB: Pattern recognition receptors in HIV transmission. *Front Immunol* 2012;3:59.
- Anton PA, *et al.*: Differential immunogenicity of vaccinia and HIV-1 components of a human recombinant vaccine in mucosal and blood compartments. *Vaccine* 2008;26:4617–4623.
- Merad M, Ginhoux F, and Collin M: Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol* 2008;8:935–947.
- Fischetti L, Barry SM, Hope TJ, and Shattock RJ: HIV-1 infection of human penile explant tissue and protection by candidate microbicides. *AIDS* 2009;23:319–328.
- Valladeau J, *et al.*: Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 2000;2:71–81.
- Cao X, *et al.*: CD1 molecules efficiently present antigen in immature dendritic cells and traffic independently of MHC class II during dendritic cell maturation. *J Immunol* 2002;169:4770–4777.
- Sugita M, Peters PJ, and Brenner MB: Pathways for lipid antigen presentation by CD1 molecules: Nowhere for intracellular pathogens to hide. *Traffic* 2000;1:295–300.
- Sugita M, Barral DC, and Brenner MB: Pathways of CD1 and lipid antigen delivery, trafficking, processing, loading, and presentation. *Curr Top Microbiol Immunol* 2007;314:143–164.
- Porcelli SA and Modlin RL: The CD1 system: Antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* 1999;17:297–329.
- Hussain LA and Lehner T: Comparative investigation of Langerhans' cells and potential receptors for HIV in oral, genitourinary and rectal epithelia. *Immunology* 1995;85:475–484.

27. Qin Q, *et al.*: Langerhans' cell density and degree of keratinization in foreskins of Chinese preschool boys and adults. *Int Urol Nephrol* 2009;41:747–753.
28. Ganor Y, *et al.*: The adult penile urethra is a novel entry site for HIV-1 that preferentially targets resident urethral macrophages. *Mucosal Immunol* 2013;6(4):776–786.
29. Bauer J, *et al.*: A strikingly constant ratio exists between Langerhans cells and other epidermal cells in human skin. A stereologic study using the optical disector method and the confocal laser scanning microscope. *J Invest Dermatol* 2001;116:313–318.
30. Ginhoux F, *et al.*: Blood-derived dermal Langerin+ dendritic cells survey the skin in the steady state. *J Exp Med* 2007;204:3133–3146.
31. Bursch LS, *et al.*: Identification of a novel population of Langerin+ dendritic cells. *J Exp Med* 2007;204:3147–3156.
32. Seré K, *et al.*: Two distinct types of Langerhans cells populate the skin during steady state and inflammation. *Immunity* 2012;37:905–916.
33. Rajkovic I, *et al.*: Differences in T-helper polarizing capability between human monocyte-derived dendritic cells and monocyte-derived Langerhans'-like cells. *Immunology* 2011;132:217–225.
34. Geissmann F, *et al.*: Transforming growth factor β 1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. *J Exp Med* 1998;187:961–966.
35. Moody DB and Porcelli SA: Intracellular pathways of CD1 antigen presentation. *Nat Rev Immunol* 2003;3:11–22.
36. Palucka K, Banchereau J, and Mellman I: Designing vaccines based on biology of human dendritic cell subsets. *Immunity* 2010;33:464–478.
37. Pavli P, Hume DA, Van De Pol E, and Doe WF: Dendritic cells, the major antigen-presenting cells of the human colonic lamina propria. *Immunology* 1993;78:132–141.
38. Pavli P, Maxwell L, Van de Pol E, and Doe F: Distribution of human colonic dendritic cells and macrophages. *Clin Exp Immunol* 1996;104:124–132.
39. Howe R, *et al.*: Evidence for dendritic cell-dependent CD4+ T helper-1 type responses to commensal bacteria in normal human intestinal lamina propria. *Clin Immunol* 2009;131:317–332.
40. Dillon SM, *et al.*: Human Intestinal lamina propria CD1c+ dendritic cells display an activated phenotype at steady state and produce IL-23 in response to TLR7/8 stimulation. *J Immunol* 2010;184:6612–6621.
41. Soilleux EJ, *et al.*: Constitutive and induced expression of DC-SIGN on dendritic cell and macrophage subpopulations *in situ* and *in vitro*. *J Leukoc Biol* 2002;71:445–457.
42. Woltman AM, *et al.*: Quantification of dendritic cell subsets in human renal tissue under normal and pathological conditions. *Kidney Int* 2007;71:1001–1008.
43. Krutzik SR, *et al.*: TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. *Nat Med* 2005;11:653–660.
44. Montoya D, *et al.*: Divergence of macrophage phagocytic and antimicrobial programs in leprosy. *Cell Host Microbe* 2009;6:343–353.
45. Coiras M, López-Huertas MR, Pérez-Olmeda M, and Alcamá J: Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nat Rev Microbiol* 2009;7:798–812.
46. Van der Vlist M and Geijtenbeek TBH: Langerin functions as an antiviral receptor on Langerhans cells. *Immunol Cell Biol* 2010;88:410–415.
47. Fahrback KM, Barry SM, Anderson MR, and Hope TJ: Enhanced cellular responses and environmental sampling within inner foreskin explants: Implications for the foreskin's role in HIV transmission. *Mucosal Immunol* 2010;3:410–418.
48. Li H, Gillis J, Johnson RP, and Reeves RK: Multifunctional plasmacytoid dendritic cells redistribute to gut tissues during SIV infection. *Immunology* 2013;140(2):244–249.
49. Kwa S, *et al.*: Plasmacytoid dendritic cells are recruited to the colorectum and contribute to immune activation during pathogenic SIV infection in rhesus macaques. *Blood* 2011;118:2763–2773.
50. McElrath MJ, *et al.*: Comprehensive assessment of HIV target cells in the distal human gut suggests increasing HIV susceptibility toward the anus. *J Acquir Immune Defic Syndr* 2013;63:263–271.

Address correspondence to:

Maria-Teresa Ochoa

Department of Dermatology

USC Keck School of Medicine

1441 Eastlake Avenue Topping Tower, Suite 3405

Los Angeles, California 90033

E-mail: maria.ochoa@med.usc.edu