2017

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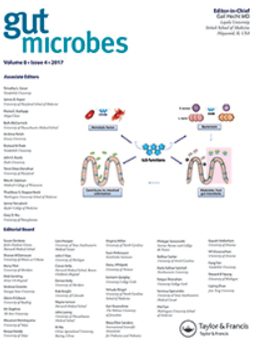

Gut Microbes

ISSN: 1949-0976 (Print) 1949-0984 (Online) Journal homepage: http://www.tandfonline.com/loi/kgmi20

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To cite this article: Kathryn A. Knoop, Jenny K. Gustafsson, Keely G. McDonald, Devesha H. Kulkarni, Rachel Kassel & Rodney D. Newberry (2017) Antibiotics promote the sampling of luminal antigens and bacteria via colonic goblet cell associated antigen passages, Gut Microbes, 8:4, 400-411, DOI: 10.1080/19490976.2017.1299846

To link to this article: https://doi.org/10.1080/19490976.2017.1299846

Accepted author version posted online: 07 Mar 2017.
Published online: 20 Mar 2017.

GUT MICROBES
2017, VOL. 8, NO. 4, 400–411
https://doi.org/10.1080/19490976.2017.1299846

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ADDENDUM

Antibiotics promote the sampling of luminal antigens and bacteria via colonic goblet cell associated antigen passages

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ABSTRACT

Bacterial translocation is defined as the passage of live bacteria from the gut lumen to distant sites. Gut commensal bacteria translocation has been attributed to 'leakiness', or 'barrier breach' of the intestinal epithelium, allowing live bacteria to cross an inappropriately permeable barrier and disseminate to distant sites. Alternatively, studies suggest dendritic cells directly capture luminal commensal bacteria and transport them to distant sites in the steady-state by extending dendrites between epithelial cells into the lumen. Recently we identified translocation of commensal gut bacteria following antibiotics was associated with the formation of goblet cell associated antigen passages (GAPs) in the colon and dependent upon goblet cells (GCs). The translocation of native gut commensal bacteria resulted in low-level inflammatory responses and potentiated mucosal damage in response to concurrent epithelial injury. Here we extend these observations and demonstrate properties of colonic GAPs and observations supporting their priority in the translocation of colonic commensal bacteria.

ARTICLE HISTORY
Received 22 November 2016
Revised 11 January 2017
Accepted 21 February 2017

KEYWORDS
antibiotics; bacterial translocation; goblet cells

At the time of their introduction, antibiotics were viewed as miracle drugs, allowing patients to survive diseases that were previously thought to be untreatable. However widespread exposure to antibiotics is now associated with multiple immune mediated disorders, which in turn have been correlated with antibiotic induced alterations of the gut microbiota.[1-6] Yet how dysbiosis of the gut microbiota confers a risk for inflammatory immune responses at local and distant sites is poorly understood. We identified an unappreciated effect of antibiotics inducing the translocation of commensal gut bacteria and inducing inflammatory responses by allowing colonic goblet cells (GCs) to form goblet cell associated antigen passages (GAPs) and deliver luminal substances, including live bacteria, to antigen presenting cells (APCs) in the colon lamina propria (LP).[7]

The gut LP contains a spectrum of myeloid APCs, including CD11b+ CD103- CX_3CR1+ APCs with features of macrophages and CD11b+ CD103+ CX_3CR1- APCs with features of dendritic cells,[8-11] which will be referred to collectively as mononuclear phagocytes (MNPs). GAPs were observed to be a major pathway delivering luminal antigens to LP-MNPs in the steady-state, as deletion of GCs or manipulation of GAPs abrogates antigen delivery to LP-MNPs as evidenced by their inability to induce T cell responses to luminal antigen.[12,13] GAPs form in response to acetylcholine (ACh) acting upon the muscarinic ACh receptor 4 (mAChR4) expressed by GCs.[13] The formation of GAPs occurs in the steady-state in the small intestine (SI), but conversely GAP formation is inhibited in the colon of SPF housed mice by GC intrinsic Myd88 dependent sensing of the microbiota.[13] Myd88 dependent microbial sensing in GCs trans-activates the epidermal growth factor receptor (EGFR) activating p42/p44 mitogen activated protein kinase (MAPK), which suppresses responses to ACh via the mAChR4[13]. Consistent with this we observed that treatment with oral antibiotics induced the formation of colonic GAPs, and

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Addendum to: Knoop KA, McDonald KG, Kulkarni DH, Newberry RD. Antibiotics promote inflammation through the translocation of native commensal colonic bacteria. *Gut* 2016; 65:1100-9

this was followed by spontaneous translocation of native colonic commensal bacteria to the colon draining mesenteric lymph node (MLN) as the gut microbiota was being restored following antibiotic cessation.[7]

This effect of antibiotics occurs following a single dose of antibiotics, during continuous sub-therapeutic antibiotic therapy, or during a period of time after cessation of therapeutic antibiotic therapy in which colonic GAPs are formed due to the loss of suppressive effects of the intact gut microbiota on GAP formation, but when the gut bacterial load is sufficient to translocate.[7] The translocation of commensal bacteria after antibiotics or in mice with reduced or absent microbiota, such as germfree mice, is a well-documented phenomena,[14-17] correlating to our finding that the presence of the normal gut flora inhibits colonic GAPs and prevents bacteria translocation. Further we observed that most but not all antibiotics evaluated induced colonic GAP formation and bacterial translocation despite equivalent alterations in the overall colonic bacterial load,[7] suggesting that some bacteria may have an enhanced capacity to inhibit GAPs and subsequent translocation and giving hope that antibiotic therapies could be tailored to mitigate this untoward effect. However the considerable questions as to which bacterial species inhibit colonic GAP formation and translocation in a given individual's gut microbiota and how antibiotics could be tailored for an individual's gut microbiota to avoid translocation will require significant more work to be answered.

Regional location and properties of colonic GAPs and translocation of commensal gut bacteria in the presence and absence of antibiotic treatment

Translocation of gut commensal bacteria to the MLN draining the SI was not observed in the absence of antibiotic therapy, and was rare in the presence of antibiotic therapy,[7] suggesting that SI GAPs, which are present independent of antibiotic treatment do not have the property of commensal bacterial translocation. The small amount of bacteria seen in a few instances following antibiotic therapy in the MLN draining the SI may have translocated via colonic GAPs as the lymph drainage patterns between the SI and portions of colon can overlap. We expanded these observations by evaluating the presence of GAPs in various regions of the colon using *in vivo* 2-photon (2P) imaging approaches and by imaging fixed tissue sections following the administration of luminal fluorescent dextran.[12,13] The latter approach allows evaluation of GAP density in regions of the GI tract that are difficult to image using the *in vivo* approach due to the abundant luminal contents, such as in the cecum, or areas that are not easily accessible for *in vivo* imaging due to anatomy, such as the sigmoid colon. Consistent with our prior observations, we found that GAPs were rare, or nearly absent, in the cecum, ascending colon, and transverse colon in the steady-state (Fig. 1A, B, D, and E). However in contrast to the proximal colonic regions, GAPs were observed in the crypts of the descending colon and sigmoid colon in the absence of antibiotic treatment (Fig. 1A, B, D, and E). The presence of GAPs in the distal colon and their absence in the proximal colon in the steady-state, may provide the basis for the ability of SPF housed mice to be tolerized to high doses of antigen in the colonic lumen in the iliac and caudal lymph nodes, which drain the distal colon segments, but not in the MLNs draining the proximal colon in the steady-state.[18]

The colonic lymphatics drain into 3 anatomically distinct lymph node (LN) populations[19,20] (Fig. 1C). The cecum and ascending colon lymphatics drain into the colonic MLN, the transverse colon lymphatics drain to a duodendopancreatic LN found behind the colon, referred to here as the colic LN, and the descending and sigmoid colon drain into the caudal LN and iliac LNs[19-21] (Fig. 1C). This lymphatic drainage pattern allows evaluation of live bacteria in the various LN populations to inform where bacteria have traversed the colonic epithelium[21,22] Despite the presence of colonic GAPs in the descending and sigmoid colon, we did not observe live commensal bacteria in the caudal or iliac LNs at steady-state (Fig. 1F). The presence of GAPs and the absence of bacterial translocation to the LN draining the distal colon may be due to the more dense and less penetrable mucus layer in the distal colon,[23] which would reduce the exposure of GCs to bacterial products and allow GAPs to form while at the same time preventing luminal bacteria from accessing the GAPs.

Following antibiotic therapy, which disrupts the luminal commensal bacteria, we observed a significant increase in GAP formation in all segments of the colon with the exception of the sigmoid colon, which showed a trend towards an increase (Fig. 1A, B, D, and E).

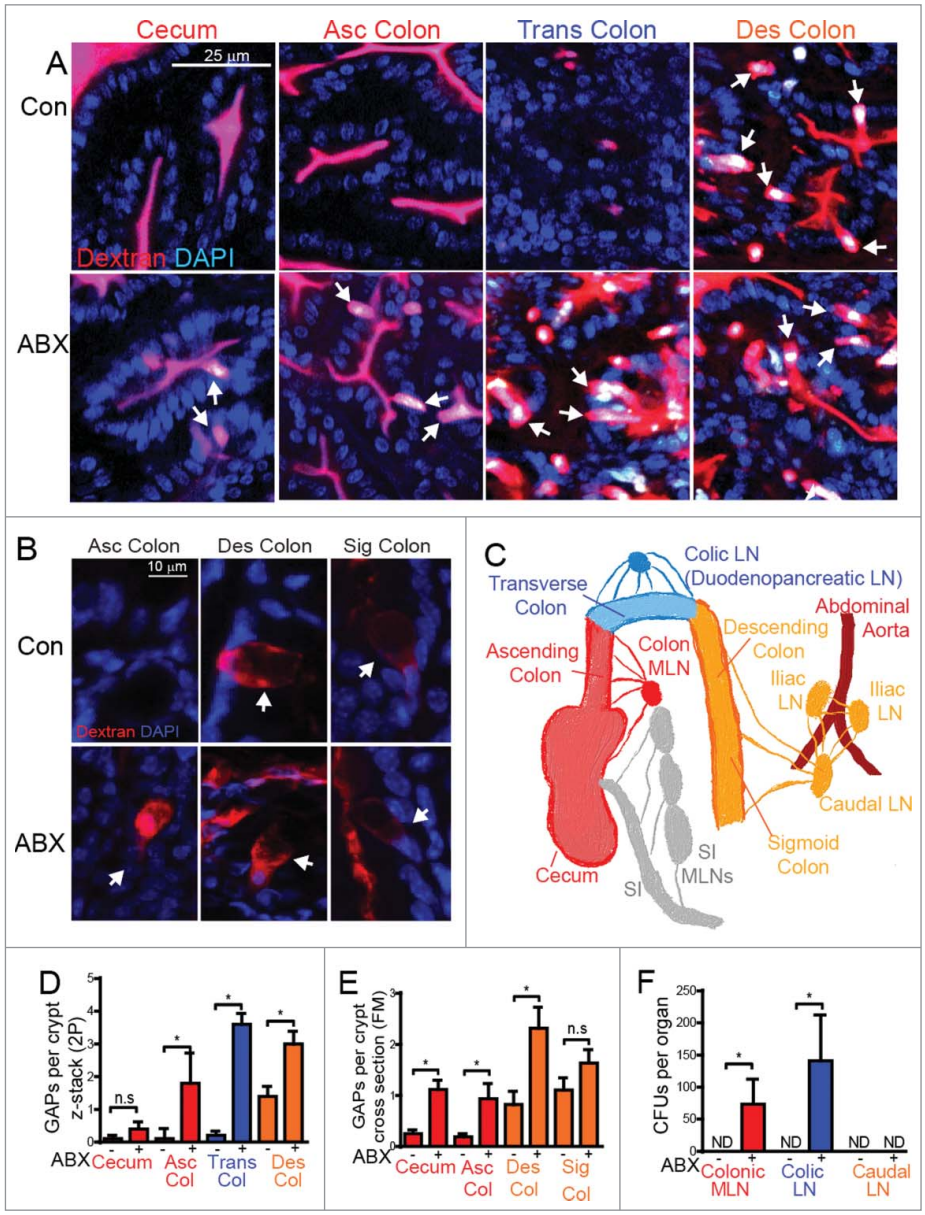

Figure 1. *Regional location of colonic GAPs and translocation of commensal gut bacteria in the presence and absence of antibiotic treatment.* (A) Mice were given regular water (top row; Con) or Ampicillin (1 g/L), metronidazole (1 g/L), neomycin(1 g/L), and vancomycin (500 mg/L) (second row; ABX) in drinking water for 1 week, and then placed on regular drinking water for 3 days.[7] For two-photon (2P) imaging, mice were injected intraluminally with 2 mg 10 kDa rhodamine-labeled dextran and 1 mg DAPI. 20 minutes later mice were imaged as described previously.[7,12,13] White arrow denotes GAPs; GCs filled with dextran. (B) After 30 minutes of fixation in 10% buffered formalin, colonic tissue with intraluminal dextran was sectioned and imaged by routine fluorescent microscopy to visualize structure of GAPs. White arrows denote GAPs. (C) Schematic summarizing the lymphatic draining pattern of the intestine. (D-E) Graphical representation of GAPs per crypt in the regions of the colon in control mice or mice following antibiotic treatment by D) 2P imaging E) or fluorescent microscopy (FM) of fixed tissue sections. Color of the bars in graphs correlates with the segment of colon draining to the corresponding LN in panel C and F. (F) Graphical representation of CFUs per LN after plating LN homogenates[7] on LB agar overnight in control mice or mice following antibiotic treatment. n = 4 mice per group, Asc colon = ascending colon, Trans colon = transverse colon, Des colon = descending colon, Sig colon = sigmoid colon, ND = not detected, ns = not significant, statistical analysis performed using a Student's t-test, $^{*} = p < 0.05$.

Imaging of fixed tissues revealed that GAPs forming in the sigmoid colon in the steady-state and those forming after antibiotic therapy appeared structurally similar (Fig. 1B). The level of GAP induction following antibiotic treatment correlated with the level of translocation of live commensal bacteria to the LNs, with the exception of the LN draining the distal colon, which did not contain bacteria in the steady-state or following

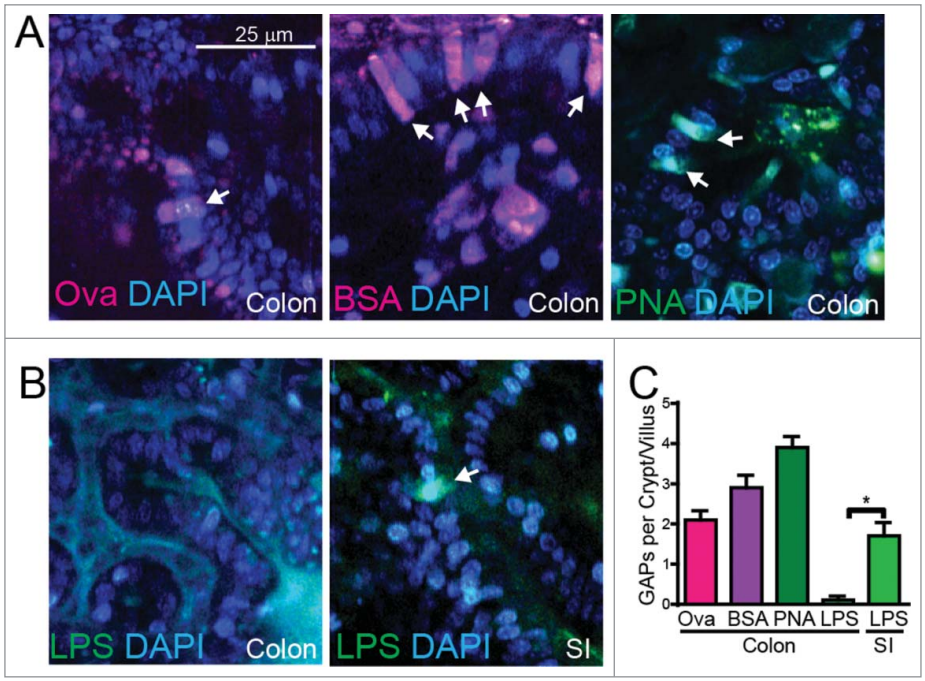

Figure 2. *Colonic GAPs acquire a variety of substances following antibiotic treatment*. (A) Representative 2-photon images of transverse colonic GAPs after antibiotic treatment filled with (left) luminal alexa-647 labeled ovalbumin (Ova), (middle) luminal alexa-647 labeled bovine serum albumin (BSA), or (right) luminal fluorescein isothiocyanate (FITC) labeled peanut agglutinin (PNA), white arrows denote GAPs. (B) Representative 2-photon images of (left) transverse colon or (right) distal ileum showing colonic GCs do not, but SI GC do fill with FITC labeled lipopolysaccharide (LPS), white arrows denote GAPs. (C) Graphical representation of GAPs per colonic crypt or SI villus filled with various proteins or lectins as assessed by 2P imaging. n = 3 mice per group, statistical analysis was performed using a t-test, * = $p < 0.05$.

antibiotic treatment (Fig. 1D-F), further supporting that a more dense mucus layer may protect the caudal LN from translocation of bacteria through the distal colon. Colonic GAPs in the proximal colon that were induced by antibiotic therapy transported a variety of luminal substances including proteins, lectins, and sugars (Fig. 2A, C); however unlike their SI counterparts, colonic GAPs did not transport LPS (Fig. 2B, C) as GC sensing of luminal LPS rapidly shut off GAPs in the colon, but not in the SI.[13]

CX3CR1+ LP-MNPs interact with GAPs in the SI and colon

CX_3CR1- (CD103+) LP-MNPs were observed to interact with SI GAPs more often that CX_3CR1+ (CD103-) LP-MNPs in the steady-state, however luminal antigen was acquired in a manner capable of inducing T cell responses by both SI LP-MNP subtypes when GAPs were robustly induced by exogenous administration of the ACh analog carbamylcholine.[12] This suggests that CX_3CR1+ LP-MNPs also acquire antigen from GAPs, but consistent with other reports are less efficient at stimulating T cell responses.[8] Furthermore CX_3CR1+ LP-MNPs interact with and acquire luminal antigen from colonic GAPs,[7,13] and are necessary for the dissemination of translocated bacteria to the MLN,[7,24] suggesting that CX_3CR1+ LP-MNPs interact with colonic GAPs to acquire luminal commensal bacteria following antibiotic therapy. We therefore evaluated if CX_3CR1+ LP-MNPs associated with GAPs in the various regions of the intestine in the steady-state and following antibiotic therapy. We observed CX_3CR1+ LP-MNPs making multiple contacts with GAPs in the SI, spanning several z-stacks (Fig. 3A) and forming contacts with 20% of the GAPs during *in vivo* imaging (Fig. 3C). We found that CX_3CR1+ LP-MNPs were removed from the epithelium in the ascending and transverse colon in the steady-state (Fig. 3D), but associated with the epithelium and were found interacting with GAPs, wrapping dendrites around GAPs, and forming contacts with >60% of GAPs in the ascending and transverse colon following antibiotic therapy (Fig. 3D, E, F). CX_3CR1+ LP-MNPs made contacts with 40% of the GAPs in the distal colon in the steady-state, but did not

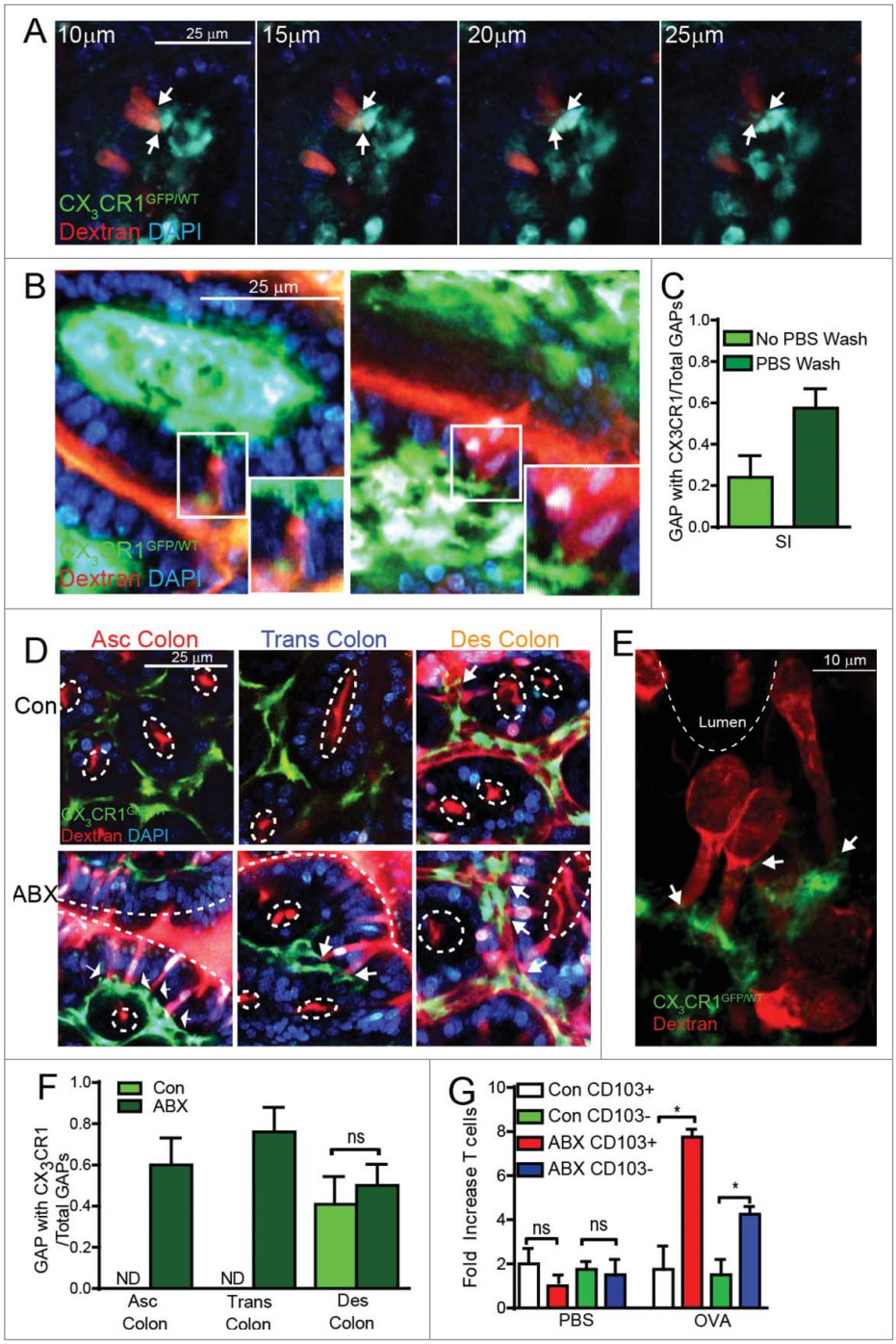

Figure 3. *CX_3CR1+ LP-MNPs interact with GAPs and acquire luminal antigen in the colon following antibiotic treatment*. (A) Serial 5μm z-stacks of a villus from a $CX_3CR1^{GFP/WT}$ reporter mouse[41] starting 10μm from the lumen acquired by *in vivo* 2P imaging after intraluminal injection with 10 kDa fluorescent dextran and DAPI; white arrows denotes CX_3CR1+ cell contacting a GAP. (B) 2P images of a villus from a $CX_3CR1^{GFP/WT}$ mouse after intraluminal injection with dextran and DAPI, inset shows higher magnification of CX_3CR1+ cell contacting a GAP. (C) Graphical representation of the ratio of GAPs being contacted by a CX_3CR1+ cell to the number of total GAPs in the SI in control mice or after washing the lumen with 5 ml of 37°C PBS to remove the luminal contents and mucus, as described in imaging approaches used by others.[27-29,33,34] (D) 2P images of the colon of a control $CX_3CR1^{GFP/WT}$ mouse (Con) or following antibiotic treatment (ABX) after intraluminal injection of dextran and DAPI, white arrows denotes CX_3CR1+ cell contact with a GAP, white dotted line indicates lumen. (E) Confocal microscopy image of $CX_3CR1^{GFP/WT}$ mouse following antibiotic treatment showing CX_3CR1+ cell contacting a dextran filled GAP (white arrow) in the transverse colon; white dotted line indicates lumen. (F) Graphical representation of the ratio of GAPs being contacted by a CX_3CR1+ cell to the number of total GAPs in the colon of control mice or after antibiotic treatment. (G) Control mice (Con) or antibiotic treated mice (ABX) were injected intraluminally with PBS or 10 mg ovalbumin (Ova); 2 hours later LP-MNPs were isolated from the colon, sorted as $CD45^+CD11c^+MHCII^+CD103^+$ cells or $CD45^+CD11c^+MHCII^+CD103^-$ cells, and cultured with sorted $CD45^+CD62L^+CD3^+CD48^+V_\alpha2^+$ Ova specific splenocytes from OTI T cell receptor transgenic mice[42] at a 1:10 ratio. Graphical representation of fold increase OTI T cells 3 d after co-culture with LP-MNPs. n = 3 mice per group, statistical analysis was performed using a Student's t-test, * = $p < 0.05$, ns = not significant, ND = not detected.

significantly increase after antibiotic treatment (Fig. 3D, F). Furthermore we found that following antibiotic treatment, CD103+ LP-MNPs and CD103- LP-MNP, which are CX_3CR1+,[7,11] acquired luminal ovalbumin in a manner able to induce naïve T cell proliferation (Fig. 3G), further supporting that CX_3CR1+ LP-MNPs can acquire luminal substances, including soluble antigens and bacteria from GAPs.[7]

Priority of GCs/GAPs in the translocation of native commensal bacteria following antibiotic therapy

Translocation of non-pathogenic luminal bacteria after antibiotic treatment is not associated with barrier breach or changes in tight junction permeability,[25] making alterations in barrier function an unlikely mediator of this event. Villous M-cells could translocate luminal bacteria to the underlying LP without altering barrier function, however M-cells have not been observed on the non-follicle bearing epithelium of the colon[26] where the translocation of commensal bacteria occurs following antibiotics,[7,15] making them unlikely participants in this process. The discovery of the ability of LP-MNPs to extend trans-epithelial dendrites (TEDs) to sample the lumen provided an explanation for how bacterial translocation might occur in the absence of epithelial damage or loss of barrier function. However several inconsistencies, including the density and regional location of TED extension[27-32] and the ability of microbial stimuli to induce TED extension while suppressing bacterial translocation,[7,24,28] suggest that TEDs may not be the mechanism for translocation of luminal commensal bacteria following antibiotic therapy. We evaluated the ability of CX_3CR1+ colonic LP-MNPs to extend TEDs in the presence and absence of antibiotic therapy. Consistent with other reports,[30] we did not observe TED extension by CX_3CR1+ colonic LP-MNPs in the steady-state or following antibiotic treatment (Fig. 4A). While CX_3CR1+ colonic LP-MNPs increased contact with the colonic epithelium and GAPs after antibiotic treatment, CX_3CR1+ dendrites were never seen crossing the epithelial barrier to contact the lumen. Thus colonic TED formation was unlikely to mediate translocation of colonic luminal bacteria.

Furthermore we found the formation of TEDs to be an exceedingly rare even in the SI of untreated mice (Fig. 4C); we observed only 2 TEDs while evaluating over 500 entire villi, from tip to base (Fig. 4E). We noted that studies of LP-MNP TED extension in the SI, rinsed the lumen of the intestine with PBS to remove the luminal contents and mucus,[27-29,33,34] a practice we did not use as mucus is a vital component of the barrier protecting the epithelium from the luminal contents.[23,35] When we removed the luminal contents and mucus by rinsing with PBS, TED extension by LP-MNPs became apparent on the tip and in the middle of SI villi (Fig. 4D) at a rate of approximately 1.5 TEDs per villus in the ileum or ~0.7 TEDs per villus combining all regions of the SI when examining over 300 villi (Fig. 4E), which is highly consistent with prior studies reporting the density and regional location of TED extension.[27,28] Often TEDs could be seen near or extending past GAPs (Fig. 4D right top, right bottom) but TEDs also formed independently of GAPs (Fig. 4D left bottom), indicating that TED formation was not dependent upon GAPs. Even after stringent washing, TEDs failed to form in the colon (Fig. 4B), corroborating the lack of TED extension in the colon in other models.[30,31]

Evaluating the density of TED extension and GAPs simultaneously within the same mouse we observed that GAPs were significantly more common than the extension of TEDs in the SI in the steady-state (~1000 fold) and in the colon following antibiotic treatment (~1000 fold; Fig. 4E). Additionally, removal of the protective mucus barrier from the epithelium before imaging increased the contacts CX_3CR1+ LP-MNPs made with SI GAPs to 60% of the GAPs (Fig. 3C), suggesting CX_3CR1+ SI LP-MNPs increase both TED extension and sampling from GAPs when the mucus barrier is disturbed. Where examined, all studies evaluating the extension of TEDs by LP-MNPs agree that they are induced by enteric pathogens.[27-29,34] Our observation that TED extension by LP-MNPs becomes more common after removal of the mucus layer and observations that epithelial Myd88 signals induce SI LP-MNPs to extend TEDs,[28] together suggest that TED extension occurs in response to epithelial stress signals. Conversely GAPs are inhibited by epithelial sensing of the microbiota in the colon.[13] While SI GAPs were not inhibited by the commensal microbiota, SI GCs expressed TLRs, MyD88, and the EGFR, and activation of this pathway by EGFR ligands can inhibit GAPS in the SI. The ability of the commensal microbiota to inhibit GAPs in the colon, but not in the SI, could be due to lower levels of expression of TLRs and/or higher expression of negative regulators

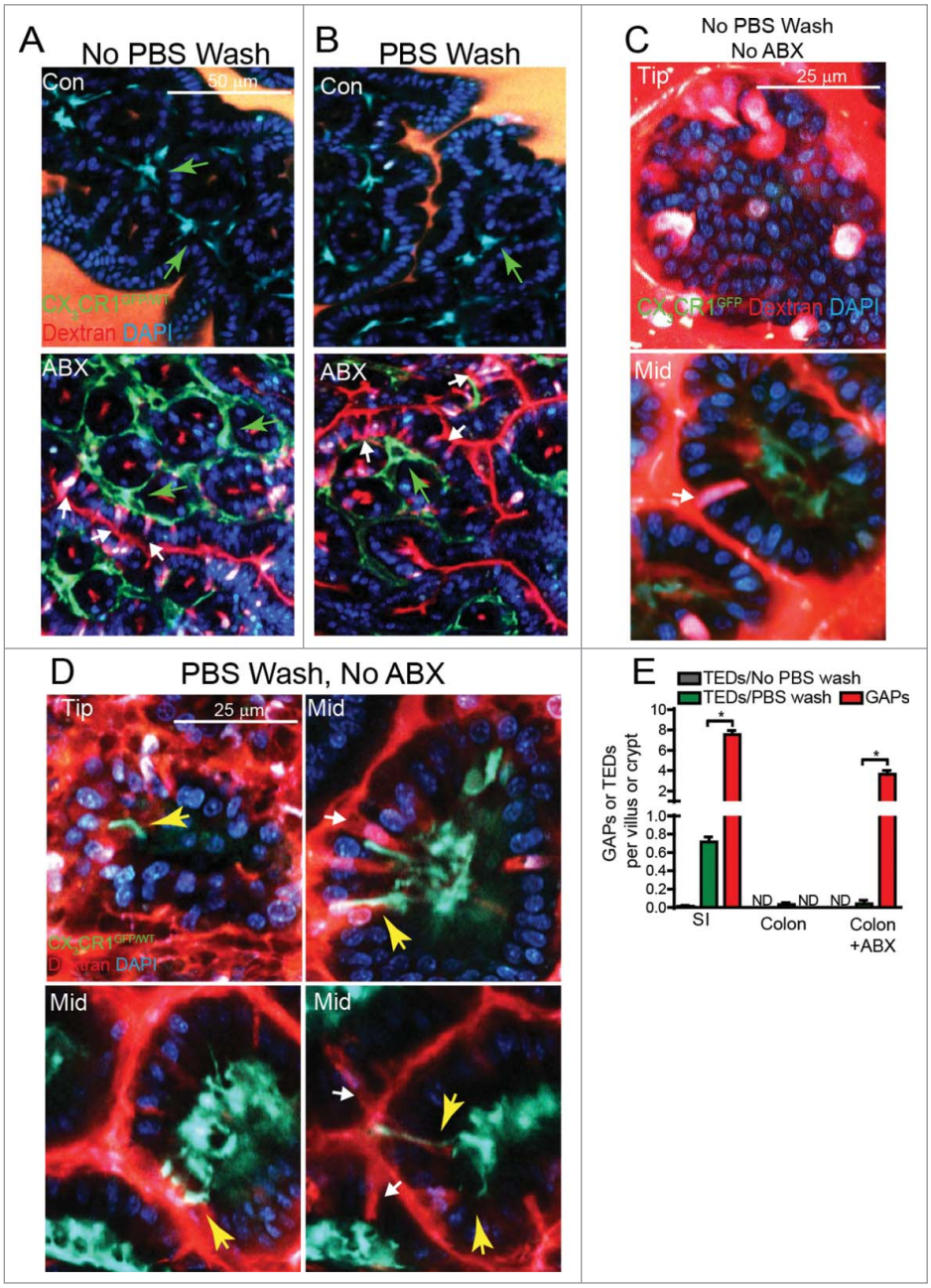

Figure 4. *GAPs are significantly more common than the extension of trans-epithelial dendrites (TEDs) by CX_3CR1+ LP-MNPs in the steady-state or following antibiotic treatment.* 2P images of the transverse colon (A) without PBS wash or (B) with PBS wash to remove luminal contents and mucus from $CX_3CR1^{GFP/WT}$ mice given drinking water alone (Con) or antibiotic treatment (ABX) and intraluminal injection of dextran and DAPI; green arrows denote CX_3CR1+ LP-MNPs, white arrows denote GAPs. (C) 2P images of the tip and mid small intestinal villus of an untreated $CX_3CR1^{GFP/WT}$ mouse after intraluminal injection of dextran and DAPI, white arrows denote GAPs. (D) 2P images of the tip and mid small intestinal villus of an untreated $CX_3CR1^{GFP/WT}$ mouse after intraluminal injection of dextran and DAPI following PBS rinsing, white arrows denote GAPs, yellow arrow denotes a TED. (E) Graphical representation of the number of TEDs, counted as $CX_3CR1^{GFP/WT}$ dendrites extending into the lumen, as delineated by the DAPI+ epithelial border, per villus or ascending colonic crypt and the number of GAPs per villus or ascending colonic crypt to show frequency of TEDs compared the frequency of GAPs. n = 3 mice per group in panel E, statistical analysis was performed using a Student's t-test, * = $p < 0.05$, ND = not detected.

of TLR signaling in SI GCs when compared with colon GCs.[13] However the preservation of this pathway in SI GCs raises the possibility that SI GAPs could be inhibited in some situations, such as enteric infection. These observations suggest that GAPs and TEDs may play opposite roles as routes for the immune system's exposure to luminal substances, with GAPs having a higher priority in homeostatic responses and TED extension by SI LP-MNPs having a higher priority during stress and infection.

Dissemination of luminal bacteria to the MLN requires sphingosine 1 phosphate receptor 1 (S1PR1)

The gut commensal microbiota restricts translocation and dissemination of commensal bacteria.[7,24] Translocation of bacteria in the colon is independent of Myd88 signals, however the carriage of bacteria to the MLN was induced by the presence of the microbiota[7,24,30] Migration of leukocytes to and from LNs is dependent on sphingosine 1 phosphate gradients and inactivation of the sphingosine

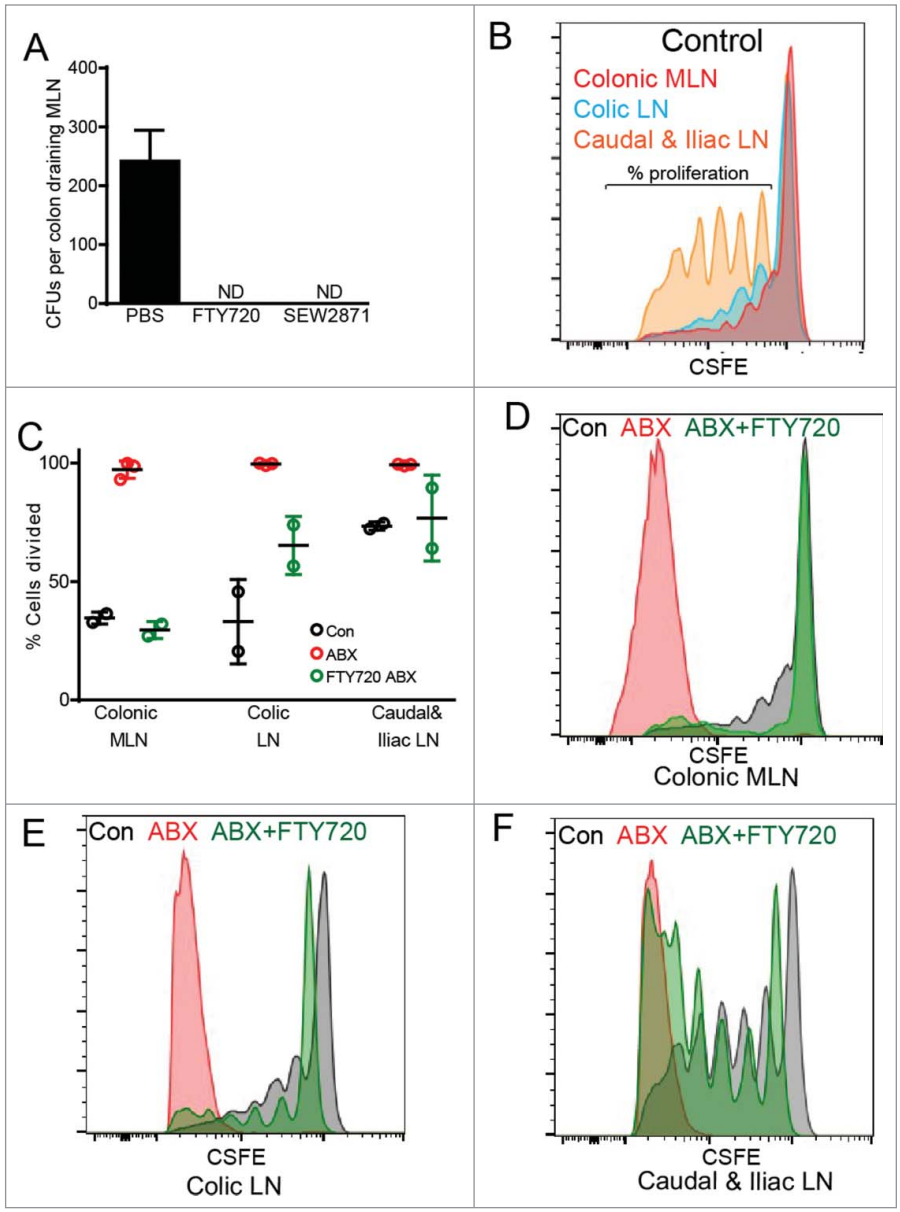

Figure 5. *Dissemination of translocated bacteria and induction of T cell responses in the LN draining the proximal colon following antibiotic therapy are dependent upon S1PR.* (A) Graphical representation of CFUs per colon draining MLN in mice following antibiotic treatment and i.p. injection with 5 mg/kg FTY720[40] or 20 mg/kg SEW2871[43] 48 hours before MLN harvest. (B-F) Mice were given normal drinking water (Control; Con) or antibiotic treatment (ABX), i.v. injected with 4.2×10^6 CFSE labeled ovalbumin (Ova) specific CD^{4+} splenic T cells from OTII T cell receptor transgenic mice, and 24 hours later given 50 mg Ova via enema and i.p. injection of vehicle or 5 mg/kg FTY720. Three days following ovalbumin administration, colon draining LN homogenates were analyzed by flow cytometry for CFSE dilution. (B) Representation flow plot of colonic draining MLN, colic LN, and caudal and iliac LN in mice not receiving antibiotics (Control). (C) Graphical representation of percent of OTII cells from the various LN and treatments that had diluted CFSE past the initial fluorescent intensity. (D-F) Representation flow plot of (D) colonic draining MLN, (E) colic LN, and (F) caudal and iliac LN in control mice and mice following antibiotic treatment with or without FTY720 injection. n = 2 or 3 mice per group, ND = not detected.

1 phosphate receptor (S1PR) inhibits circulation of lymphocytes.[36] Intestinal APCs express the S1PR,[37] and inhibition of S1PR in clinical trials is an effective treatment of IBD and colitis,[38,39] though the mechanism of action is incompletely understood. Additionally, it has been shown CX_3CR1+ MNPs require S1PR expression for trafficking of cells carrying pathogenic bacteria to secondary LNs in a model of Yersinia infection.[40] Pan S1PR inactivation with FTY720 or selective S1PR1 inactivation with SEW2871 inhibited bacterial translocation to the colon draining MLN (Fig. 5A), suggesting that S1PR blockade might prevent inflammatory responses in the MLN.

Antibiotic therapy induces T cell responses to luminal antigens in the colon draining LN

We previously observed that following antibiotic therapy, inflammatory cytokine responses were seen in the colon draining MLN. These inflammatory responses could be due to innate immune responses to bacteria and bacterial products as well as T cell responses to luminal antigens delivered by colonic GAPs. We evaluated the ability to induce antigen specific immune responses in the colon draining LNs following antibiotic therapy. In the absence of antibiotic therapy, little to no antigen specific T cell proliferation in response to luminal antigen was observed in LN draining the cecum, ascending colon (colonic MLN) and transverse colon (colic LN), while proliferation was observed in the LNs draining the descending colon and sigmoid colon (caudal and iliac LN; Fig. 5B), correlating with presence of GAPs in the descending colon and sigmoid colon and their absence in proximal regions of the colon in the steady-state (Fig. 1A, C, D and E). This also is consistent with GAPs as a mechanism delivering antigen to the caudal and iliac LNs to induce tolerance to rectally administered antigens.[18] In contrast, following antibiotic therapy, robust T cell proliferation to luminal antigen was observed in the LNs draining all segments of the colon (Fig. 5C-F), correlating with the presence of GAPs in all segments of the colon after antibiotic treatment (Fig. 1A, C, D and E). This suggests that the inflammatory cytokines we observed to be produced in the colon draining LN[7] may influence naïve T cell differentiation to promote inflammatory responses to otherwise harmless luminal antigens encountered in the setting of bacterial translocation following antibiotic therapy.

Treatment with FTY720 to inhibit S1PR dependent migration at the time of the luminal OVA administration almost completely abrogated antigen specific T cell proliferation in the LNs draining the cecum, ascending colon and transverse colon (colonic MLN and colic LN), while only modestly affecting antigen specific T cell proliferation in the LNs draining the descending colon and sigmoid colon (caudal and iliac LN), which resembled proliferation to luminal antigen untreated mice (Fig. 5C-F). This suggests that the colonic LP-MNPs acquiring luminal antigen and inducing robust T cell responses in the MLNs following antibiotic therapy require S1PR to migrate to the MLN. This also suggests that a second LP-MNP population may acquire luminal antigens in the distal colon and carry them to the LN to induce more moderate proliferative responses in an S1PR independent manner in the absence of antibiotic therapy. Our observations might also indicate that the beneficial effect S1PR inhibitors have on colitis are in part due to inhibition of LP-MNP trafficking to the LN and subsequent inhibition of T cell responses.

Conclusion

The translocation of bacteria across the intestinal epithelium following antibiotic treatment is a well recognized and, until now, enigmatic process. Here we show a window of time following antibiotics where the colonic microbiota is permissive to the formation of colonic GAPs. Once formed, colonic GAPs can deliver a variety of substances, including protein antigens and live commensal bacteria. CD103+ and CX_3CR1+ LP-MNPs interact with GAPs to access luminal antigen, while commensal bacteria are primarily acquired by CX_3CR1+ LP-MNPs.[7] After acquiring commensal bacteria and luminal antigens LP-MNPs traffic to the draining LNs promoting an inflammatory environment, which could result in inflammatory T cell responses to otherwise innocuous antigens. It is not known if inflammatory responses are induced in the LN draining the distal colonic segments following antibiotic therapy, which may be an exception to this sequence of events, as the distal colon GAPs are present in the steady-state and bacterial translocation to the LNs draining the distal colon was not observed in the steady-state or following

antibiotic treatment. Alterations in the gut microbiota and antibiotic use are increasingly being associated with inflammatory disorders. The observations presented here demonstrate that inflammatory responses following antibiotic use occur in part due to translocation of gut bacteria via GAPs and suggest that strategies such as manipulation of the gut microbiota following antibiotic therapy to prevent translocation or blockade of LP-MNP trafficking to prevent bacterial dissemination may be approaches to mitigate antibiotic associated inflammatory responses.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors would like to thank Dr. Mark J. Miller for insightful critiques and assistance with 2P imaging and analysis. The 2P imaging was performed at the *In Vivo* Imaging Core at Washington University School of Medicine. The High Speed Cell Sorter Core at the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, MO. provided flow cytometric cell sorting services. The Siteman Cancer Center is supported in part by NCI Cancer Center Support Grant P30 CA91842. Studies were supported by the Washington University Digestive Diseases Research Center Core P30 DK052574, DK097317-RDN, Children's Discovery Institute Grant MD-II-2015–481–RDN, AI112626–RDN, AGA Providence Food Intolerance Award–RDN, and DK097893-KAK.

Funding

Work supported by NIH grants P30 CA91842, P30 DK052574, DK097317-RDN, AI112626–RDN AI131342-RDN, Children's Discovery Institute Grant MD-II-2015–481–RDN, AGA Providence Food Intolerance Award–RDN, and DK097893-KAK

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