Microbial translocation in HIV infection: causes, consequences and treatment opportunities

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Abstract | Systemic immune activation is increased in HIV-infected individuals, even in the setting of virus suppression with antiretroviral therapy. Although numerous factors may contribute, microbial products have recently emerged as potential drivers of this immune activation. In this Review, we describe the intestinal damage that occurs in HIV infection, the evidence for translocation of microbial products into the systemic circulation and the pathways by which these products activate the immune system. We also discuss novel therapies that disrupt the translocation of microbial products and the downstream effects of microbial translocation.

Antiretroviral therapy (ART) has markedly improved survival in HIV-infected individuals. Nevertheless, they remain at increased risk of morbidity and mortality owing to diseases such as cardiovascular disease, malignancy and osteoporosis[1]. When compared with age-matched controls, HIV-infected individuals are also at an increased risk of non-infectious co-morbidities, including hypertension, type 2 diabetes, bone fractures and renal failure[1]. Although the exact mechanisms responsible for this increased risk are not entirely understood, in HIV-uninfected individuals these diseases are associated with increased systemic immune activation and inflammation[2–4].

Systemic immune activation in HIV-infected people is characterized by high T cell turnover[5], B cell and T cell activation[6], and raised levels of pro-inflammatory and profibrotic mediators[7–9]. Importantly, immune activation persists even in virologically suppressed individuals on ART[10], and their risk of disease progression is independent of viral load, suggesting that factors other than virus replication may have a role.

Microbial translocation — the translocation of commensal microbial products from the intestinal lumen into the systemic circulation in the absence of overt bacteraemia — has recently been suggested to have a key role in driving this persistent immune activation in individuals with chronic HIV infection. Microbial translocation occurs even in healthy individuals but increases with damage to the intestinal barrier[11] (TABLE 1). These bacterial and fungal products may include peptidoglycan[12], lipoteichoic acid[13], lipopolysaccharide (LPS)[14], flagellin[14], ribosomal DNA (rDNA)[15] and unmethylated CpG-containing DNA[16]. These products elicit potent pro-inflammatory responses by activating a number of receptors: nucleotide-binding oligomerization domain 1 (NOD1) and NOD2, as well as Toll-like receptor 2 (TLR2), TLR4, TLR5, TLR6 and TLR9, which are expressed by many cell types, not solely the archetypal innate immune cells (BOX 1). In innate immune cells such as monocytes, macrophages and dendritic cells, binding of these receptors to microbial products activates a signalling cascade, leading to the production of the pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, tumour necrosis factor (TNF) and type I interferons (IFNs; including IFNα and IFNβ). Although these responses may be beneficial, if not essential, to the host in the setting of acute infections, they may contribute to disease pathology in numerous situations, including chronic HIV infection.

Pro-inflammatory cytokines such as IL-1β, IL-6 and TNF can induce inflammation and tissue damage in the vasculature, as well as the proliferation of local monocytes or macrophages and smooth muscle cells, resulting in cardiovascular disease[2]. They also increase the proliferation of and impair apoptosis of pre-malignant cells and malignant cells[17], and stimulate bone resorption, which leads to osteoporosis[18].

In this Review, we discuss evidence showing that intestinal damage precipitated by HIV infection leads to microbial translocation, which in turn is associated with systemic immune activation and disease progression. We also review the effects of ART on microbial

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doi:10.1038/nrmicro2848
Published online
13 August 2012

Table 1 | **Microbial translocation in disease**

Disease or infection	Potential cause of translocation	Effect	Refs
Inflammatory bowel disease	• Loss of immune tolerance to commensal organisms owing to altered pathogen recognition and processing • Heightened immune responses, leading to inflammation and epithelial loss	Increased disease activity	161–163
Excessive alcohol consumption	Cirrhosis (shunting from the portal circulation and impaired Kupffer cell function)	Hepatic inflammation and fibrosis	103,164
Hepatitis B and C	Cirrhosis (shunting from the portal circulation and impaired Kupffer cell function)	Hepatic inflammation and fibrosis	102
Dengue virus	Epithelial cell loss caused by local inflammation	Increased disease severity	165
Visceral leishmaniasis	Systemic and intestinal $CD4^+$ T cell depletion or parasitization of intestinal epithelial cells	Increased immune activation and disease severity	166
Coeliac disease	Heightened immune response to dietary gluten proteins, leading to inflammation, epithelial loss and altered commensal flora	Increased local inflammation and disease severity	75,167

translocation and describe novel therapies that impede microbial translocation during HIV infection and the downstream effects of microbial translocation.

Evidence of microbial translocation

Rhesus macaques can be infected with simian immunodeficiency virus (SIV), resulting in $CD4^+$ T cell depletion and progression to AIDS with similar pathology to HIV-infected humans[19]. By contrast, sooty mangabees, which evolved with SIV, can become infected with SIV but do not show $CD4^+$ T cell depletion and do not develop AIDS[19]. In rhesus macaques with chronic SIV infection, increased commensal *Escherichia coli* protein deposition (as detected by immunohistochemistry) occurs in both the colonic lamina propria and the liver, which drains the gastrointestinal tract and clears microbial products. *E. coli* protein deposition even occurs in the distal lymph nodes, indicating systemic microbial translocation[20]. However, sooty mangabees infected with SIV do not have increased local or distal *E. coli* protein deposition. Thus, the host that progresses to AIDS has increased microbial translocation, whereas the host that does not develop AIDS when infected with SIV does not show this increase. As HIV-infected humans have intestinal damage and microbial translocation similar to that observed in SIV-infected rhesus macaques, this dissemination of commensal microbial products to the lamina propria, liver and distal lymph nodes probably also occurs in individuals with HIV infection.

Indeed, in adults and children from North America, Europe, Australia and Africa with chronic HIV infection, systemic LPS levels are increased[8,21–37]. However, some studies have not identified an association between HIV infection and high LPS levels[9,38–40]. The discrepancy in the data could be explained by numerous factors: sample size, differences in sample type (serum versus plasma), collection and processing (including antiseptic and anticoagulant used), differences in assay technique, inhibition or degradation of LPS by circulating proteins and enzymes, demographics and recruitment methods of the HIV-uninfected individuals, and fasting status of the subjects[41,42]. An increase in triglyceride levels following food consumption interferes with LPS detection, and numerous plasma components bind LPS, including LPS-binding protein (LBP), endotoxin core antibody (EndoCAb) and high-density lipoprotein (HDL)[41,42].

By contrast, all studies have found increased levels of soluble CD14 (sCD14) in HIV-infected individuals. sCD14, which is shed or secreted by innate immune cells such as monocytes, binds LPS in complex with LBP, thereby serving as a marker of LPS-induced monocyte or macrophage activation, and it is also thought to enhance responses to LPS[43,44]. Plasma sCD14 levels are increased in children and adults infected with HIV regardless of their country of origin[8,9,21,22,25,27,29,32,33,35,40,45–49]. The relationship between sCD14 and LPS is complex in HIV-infected individuals; high sCD14 levels have been associated with high LPS levels[22,27,29,33], low LPS levels[35,38,50] or neither[8]. This variability may be due to the difficulties in measuring plasma LPS, HIV disease severity, the presence and duration of ART (LPS and sCD14 change at different rates on initiation and cessation of ART[24,51]), genetic factors[52] and the intestinal microbiota[53].

Other markers of microbial translocation are also increased in HIV-infected individuals. Bacterial 16S rDNA levels are higher in HIV-infected subjects than in controls. They also correlate with LPS levels[15,54] and are more frequently detectable in immunologic non-responders than in immunologic responders[47]. However, the sensitivity and specificity of this assay is frequently compromised by contaminating bacterial DNA in commercially prepared *Taq* polymerase and the environment[55] and by the presence of plasma DNases. Moreover, levels of flagellin-specific antibodies and LBP are increased in HIV-infected subjects compared with controls[22,56]. Furthermore, levels of EndoCAb are decreased in HIV infection[8,22,40], which is consistent with their consumption by LPS scavenging.

Lamina propria
Connective tissue that underlies the epithelium of the mucosa and contains various myeloid and lymphoid cells, including macrophages, dendritic cells, T cells and B cells.

Box 1 | **Monocyte and macrophage activation by microbial products**

Numerous pattern-recognition receptors (PRRs) are involved in the recognition of microbial components, which are also known as pathogen-associated molecular patterns (PAMPs)[158]. Toll-like receptors (TLRs) also recognize various PAMPs (FIG. 3); these transmembrane receptors are located on the cell surface (TLR1, TLR2, TLR4, TLR5 and TLR6) or on intracellular vesicles such as endosomes and lysosomes (TLR3, TLR7, TLR8 and TLR9). Lipoteichoic acid and lipoproteins from Gram-positive bacteria are recognized by TLR2 dimerized with TLR1 or TLR6. Lipopolysaccharide from Gram-negative bacteria is recognized by TLR4, and bacterial flagellin by TLR5. The four endosomal TLRs recognize nucleic acid components of viruses and bacteria. Unmethylated CpG, which is characteristic of bacterial and viral DNA, is recognized by TLR9. TLR3 recognizes double-stranded viral RNA, and TLR7 and TLR8 recognize single-stranded viral RNA. Peptidoglycan, which is expressed to a much higher extent by Gram-positive compared with Gram-negative bacteria, is recognized by TLR2 according to some reports, and also by a different type of PRR, intracellular nucleotide-binding oligomerization domain (NOD)[159]. NOD1, NOD2 and all TLRs except TLR3 activate the transcription factor nuclear factor-κB (NF-κB) and mitogen-activated protein kinases through the adaptor molecule myeloid differentiation primary-response protein 88 (MYD88), leading to the production of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and tumour necrosis factor (TNF)[158,159]. In addition, TLR3 and TLR4 activate the transcription factors NF-κB and interferon regulatory factor 3 (IRF3) through TIR-domain-containing adaptor protein inducing IFNβ (TRIF; also known as TICAM1), resulting in the production of pro-inflammatory cytokines and type I interferons[158].

Studies have also found alterations in the bacterial content of stools and the translocating commensal microbiota, which may contribute to increased immune activation. In a recent study, a higher percentage of circulating activated $CD8^+$ T cells was found in subjects with high fractions of stool Bacteroidales, and fewer $CD4^+$ T cells were found in the duodenum of individuals with high fractions of stool Enterobacteriales[57]. Furthermore, immunologic non-responders had reduced levels of plasma 16S rRNA from Lactobacillaceae and Pseudomonadaceae before and after ART compared with immunologic responders[48]. Thus, alterations in the composition of the commensal microbiota may contribute to the increased immune activation observed in HIV-infected individuals.

Causes of microbial translocation

Microbial translocation increases following damage to the intestinal barrier in many diseases. In addition to epithelial damage, systemic microbial translocation in HIV-infected individuals may result from loss of T helper 17 cells (T_H17 cells) and decreased clearance of microbial products by phagocytes.

Disruption of the epithelial barrier. Intestinal epithelial damage, caused by loss of intestinal epithelial cells (enterocytes) and disruption of tight junctions between the cells (FIG. 1), may lead to increased microbial translocation in many diseases, including HIV infection (TABLE 1).

Indeed, structural and immunological damage to the intestinal tract in individuals with HIV infection was described early in the epidemic. In addition to diarrhoea and weight loss, individuals with AIDS had markedly increased intestinal permeability (as shown by tests measuring absorption of D-xylose and fat[58]) and increased villous atrophy (as indicated by increased urinary lactulose/mannitol ratios), leading to malabsorption of nutrients and diarrhoea[59]. Indeed, jejunal biopsies revealed partial villous atrophy with crypt hyperplasia and decreased immunoglobulin A (IgA)-producing plasma cells, which is consistent with underlying B cell dysfunction in HIV-infected individuals and decreased IgA concentrations in the intestinal lumen[58,60,61]. These changes were noted in the absence of detectable bacterial, viral or parasitic infection. Bacterial overgrowth in the small intestine has also been reported in subjects with AIDS, which could further perpetuate the increased intestinal permeability[62,63].

Recent studies have found further evidence of damage to the intestinal epithelium in HIV-infected individuals. Citrulline levels are decreased, indicating loss of small intestinal enterocytes, which produce most serum citrulline[64]. Low citrulline levels correlate with a decrease in the villous height/crypt depth ratio in the small intestine, which reflects villous atrophy and increased stem cell proliferation in the crypt and is associated with malabsorption[65,66]. Moreover, HIV-infected individuals, regardless of whether they have received ART and whether they show virologic suppression, have increased plasma levels of intestinal fatty acid binding protein (I-FABP)[8], which is made exclusively by enterocytes and is released into the bloodstream on enterocyte loss. In patients infected with HIV, abnormal enterocyte differentiation or organization has been observed[65], which is possibly due to increased intraepithelial calcium concentrations[67] and impaired sodium glucose co-transport (sodium glucose co-transport protects against LPS-associated apoptosis)[68]; such abnormalities may contribute to enterocyte loss.

Increased enterocyte apoptosis has also been observed during acute HIV infection[69] (FIG. 1b). Loss of enterocytes may be caused by the virus itself[65,67,70–72], potentially owing to decreased glucose uptake by enterocytes mediated by the HIV protein Tat[73] or gp120-induced microtubule disruption[67]. Bystander effects may also contribute to enterocyte loss. For example, in coeliac disease, infiltration of $CD8^+$ T cells has been associated with increased enterocyte apoptosis[73] and the development of villous atrophy[74,75]. Enterocyte loss may also be caused by damage inflicted by pro-inflammatory cytokines such as TNF, as occurs in inflammatory bowel disease[76]. By contrast, subjects with low C-reactive protein (CRP) levels, who show low degrees of inflammation, have high citrulline levels, reflecting preservation of enterocytes[77]. Thus, increased local and systemic inflammation is associated with enterocyte loss in HIV infection.

Loss of enterocytes results in loss of epithelial tight junctions (FIG. 1b), as evidenced by the discontinuous staining of the tight junction protein claudin 3 in rhesus macaques with chronic SIV infection[20]. Tight junction loss occurs within 14 days post-infection[20] and leads to increased epithelial barrier permeability. Furthermore, the expression of genes that induce epithelial barrier maintenance is decreased in acute HIV infection[78]. Such loss of tight junctions may facilitate the translocation of microbial products. Indeed, sites with epithelial barrier disruption have an increased density of staining for *E. coli* proteins in the underlying lamina propria[20]. Increased expression of syndecan 1, to which bacteria

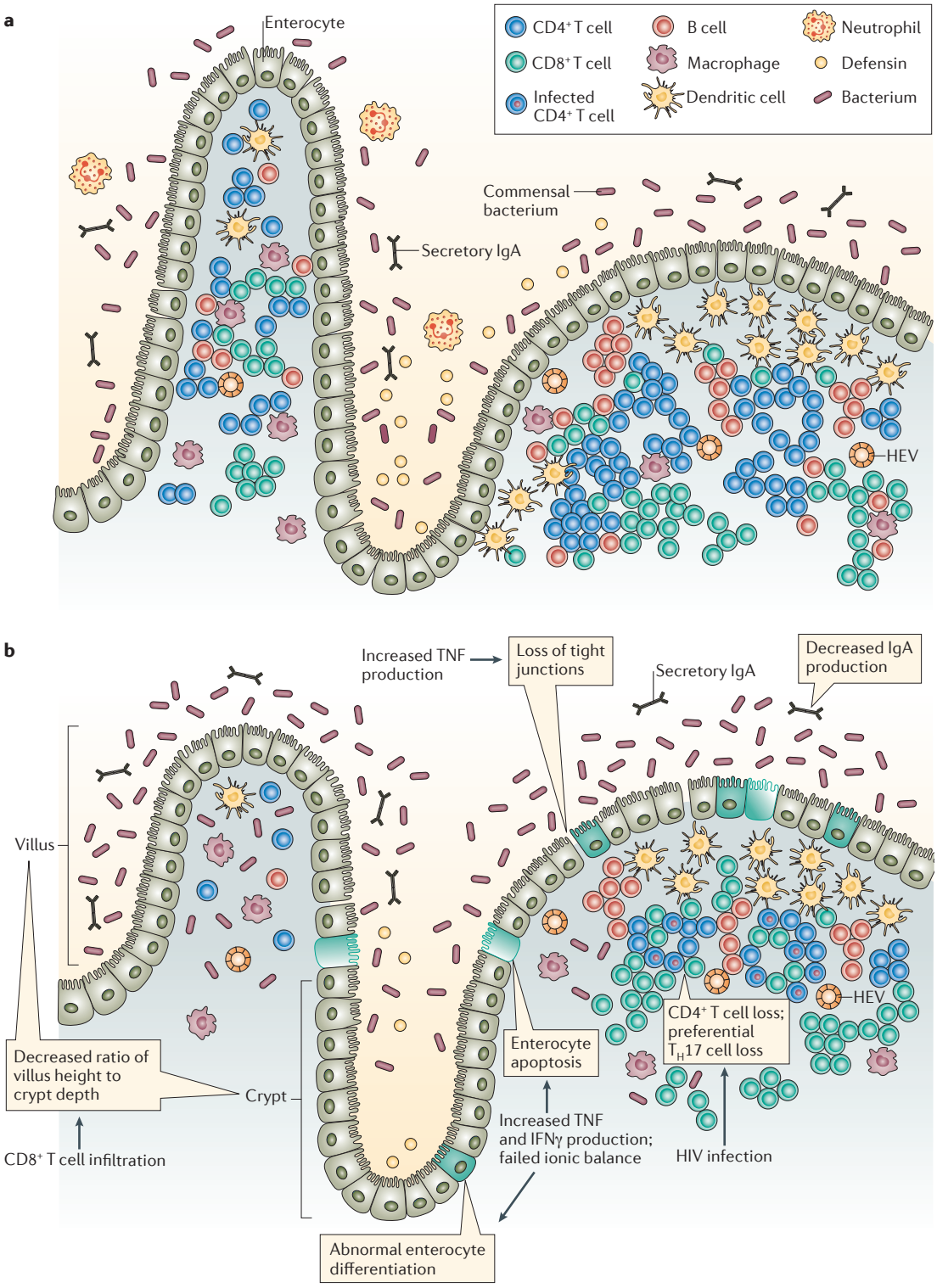
T helper 17 cells
(T_H17 cells). A subset of $CD4^+$ T helper cells that produce interleukin-17 and that are thought to be important in inflammatory and autoimmune diseases.

Tight junctions
Connections between individual epithelial cells that form a diffusion barrier between the underlying tissue layer and the extracellular environment.

Villous atrophy
Loss of enterocytes lining the villi that protrude into the small intestinal lumen.

Crypt hyperplasia
Increased proliferation of enterocytes in the crypts of the small intestine.

Citrulline
Metabolite of glutamine and arginine that is synthesized exclusively by small intestine enterocytes.

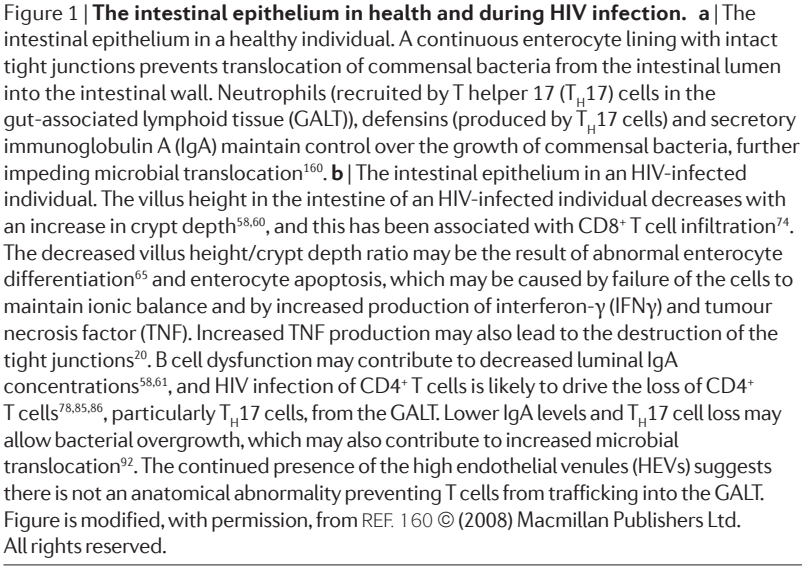


Coeliac disease
A chronic inflammatory condition of the upper small intestine in humans that is caused by immunological hypersensitivity to gliadin, a component of wheat gluten. It often occurs in infants during the introduction to solid foods. It causes severe villus atrophy, which can lead to malabsorption and malnutrition if gluten-containing foods are not removed from the diet.

C-reactive protein
(CRP). An acute-phase reactant protein produced in the liver. The concentration of CRP in plasma increases during inflammation.

can bind, at sites with epithelial barrier disruption may facilitate this microbial translocation[79].

Loss of T_H17 cells. Depletion of $CD4^+$ T cells is greater in the gastrointestinal tract than in blood or lymph nodes during HIV infection[80–84]. C-C chemokine receptor type 5 $(CCR5)^+$ $CD4^+$ T cells are depleted probably because they become directly infected by the virus[78,85,86], as CCR5 acts as a receptor for HIV virions[87]. On the basis of studies on rhesus macaques, depletion of $CD4^+$ T cells from the gastrointestinal tract occurs primarily during acute infection[88,89]. Of these T cells, T_H17 cells, which express both CCR5 and the gut homing marker CCR6, are preferentially depleted[21,84,90,91], probably because they are more susceptible to HIV infection than $CCR5^+CCR6^-$ cells, although the exact mechanism underlying this



◀ Figure 1 | **The intestinal epithelium in health and during HIV infection. a** | The intestinal epithelium in a healthy individual. A continuous enterocyte lining with intact tight junctions prevents translocation of commensal bacteria from the intestinal lumen into the intestinal wall. Neutrophils (recruited by T helper 17 (T_H17) cells in the gut-associated lymphoid tissue (GALT)), defensins (produced by T_H17 cells) and secretory immunoglobulin A (IgA) maintain control over the growth of commensal bacteria, further impeding microbial translocation[160]. **b** | The intestinal epithelium in an HIV-infected individual. The villus height in the intestine of an HIV-infected individual decreases with an increase in crypt depth[58,60], and this has been associated with $CD8^+$ T cell infiltration[74]. The decreased villus height/crypt depth ratio may be the result of abnormal enterocyte differentiation[65] and enterocyte apoptosis, which may be caused by failure of the cells to maintain ionic balance and by increased production of interferon-γ (IFNγ) and tumour necrosis factor (TNF). Increased TNF production may also lead to the destruction of the tight junctions[20]. B cell dysfunction may contribute to decreased luminal IgA concentrations[58,61], and HIV infection of $CD4^+$ T cells is likely to drive the loss of $CD4^+$ T cells[78,85,86], particularly T_H17 cells, from the GALT. Lower IgA levels and T_H17 cell loss may allow bacterial overgrowth, which may also contribute to increased microbial translocation[92]. The continued presence of the high endothelial venules (HEVs) suggests there is not an anatomical abnormality preventing T cells from trafficking into the GALT. Figure is modified, with permission, from REF. 160 © (2008) Macmillan Publishers Ltd. All rights reserved.

preferential infection remains unclear[91]. Indeed, higher viraemia was associated with greater T_H17 cell depletion from the gut-associated lymphoid tissue (GALT)[21,90] (FIG. 1b). Interestingly, T_H17 cells are not lost from the GALT of SIV-infected sooty mangabees, which, as noted above, do not have increased microbial translocation and do not develop AIDS[84].

T_H17 cells promote neutrophil recruitment and the production of antimicrobial peptides such as defensins and are thus thought to control extracellular bacteria and fungi. Therefore, loss of T_H17 cells may result in microbial overgrowth[92]. Furthermore, T_H17 cells produce IL-22, which enhances epithelial regeneration[93], so their loss may also cause impaired mucosal healing and consequently increased intestinal permeability and microbial translocation. Indeed, lower T_H17 cell frequencies in sigmoid biopsy samples from HIV-infected individuals correlate with higher plasma LPS levels, which in turn correlate with an increased HIV proviral reservoir in the gut[21]. Thus, microbial translocation is associated with both intestinal T_H17 cell depletion and increased HIV levels in the intestine.

In addition, the ratio of T_H17 cells to regulatory T cells (T_{Reg} cells) is decreased in both peripheral blood and rectosigmoid biopsy samples from individuals with HIV infection[94]. The low T_H17/T_{Reg} ratio in these individuals correlates with high plasma levels of 16S rDNA, which are indicative of microbial translocation, and with a higher frequency of activated $CD8^+$ T cells, which is one of the strongest predictors of mortality and low $CD4^+$ T cell reconstitution after starting ART[10,95]. However, it is important to note that the data are conflicting as to whether T_{Reg} cells are increased or decreased in HIV infection, probably owing to differences in populations, the markers used to characterize T_{Reg} cells, the sites examined and whether frequencies or absolute numbers were measured[96,97].

This change in T_H17/T_{Reg} ratios is associated with the induction of the immunomodulatory enzyme indoleamine 2,3-dioxygenase 1 (IDO1), which is an intracellular haem-containing enzyme that catalyses the oxidative catabolism of tryptophan to kynurenine (FIG. 2). Increased concentrations of 3-hydroxyanthranilic acid (a consequence of IDO1 upregulation) result in decreased natural killer (NK) cell production of IL-17 and IL-22. These two cytokines, which control extracellular bacteria and promote mucosal repair, are already decreased owing to T_H17 cell depletion[98]. IDO1 can be upregulated by type I IFNs and TLR agonists such as LPS and bacterial and viral DNA (BOX 1). In HIV-infected individuals, IDO1 induction is associated with increased levels of LPS and sCD14, increased $CD8^+$ T cell activation, a greater $CD4^+$ T cell decline and a reduction in $CD4^+$ T cell counts[94]. Thus, increased microbial translocation in HIV infection (a consequence of early intestinal damage and T_H17 cell loss) and systemic immune activation may further perpetuate bacterial overgrowth by inducing the production of the tryptophan catabolite 3-hydroxyanthranilic acid. This, in turn, increases T_{Reg} cell frequency to counteract the heightened immune activation as a negative feedback mechanism, drives further T_H17 cell depletion, and decreases IL-17 and IL-22 production by NK cells.

Decreased clearance of bacterial products. Microbial products drain from the gastrointestinal tract into the portal vein, which delivers these products to the liver[99]. There, Kupffer cells and hepatocytes recognize microbial components through TLRs and clear most of the LPS draining from the intestine[100]. However, Kupffer cell density is decreased in those individuals who are co-infected with HIV and hepatitis C virus and have low $CD4^+$ T cell counts; this lower Kupffer cell density possibly leads to reduced microbial product clearance and the increased LPS levels observed in co-infected individuals[33,101]. Indeed, individuals with hepatitis B or hepatitis C virus infection in the absence of HIV infection have increased microbial translocation, and greater systemic LPS-induced monocyte activation predicts cirrhosis and progression to end-stage liver disease in these patients[102]. Similarly, subjects with alcohol-induced cirrhosis have increased microbial translocation compared with heavy alcohol consumers without cirrhosis[103]. Recently, non-cirrhotic portal hypertension[104,105], liver fibrosis[106] and decreased protein synthesis by the liver[105] have been described in HIV-infected individuals, suggesting that HIV alone may be associated with impaired hepatic architecture and function, shunting blood away from Kupffer cells and decreasing the synthesis of proteins involved in LPS clearance. As noted above, in the setting of hepatitis virus infection, fibrosis is associated with increased sCD14 levels[102]. Thus, hepatic damage, regardless of the cause, may induce increased microbial translocation, resulting in hepatic inflammation. This would lead to increased hepatic fibrosis, further perpetuating the cycle.

HIV-infected subjects also have lower frequencies of intestinal $CD13^+$ myelomonocytic cells, including dendritic cells, macrophages and granulocytes, compared with HIV-uninfected individuals[84], which would further impair the removal of microbial products from

Gut-associated lymphoid tissue
(GALT). Lymphoid structures and aggregates associated with the intestinal mucosa.

Sigmoid
Terminal part of the colon adjacent to the rectum.

Regulatory T cells
(T_{Reg} cells). A subset of $CD4^+$ T helper cells that can suppress the responses of other T cells.

Kupffer cells
The macrophages of the liver. These cells are derived from blood monocytes, and they phagocytose particles, including bacteria and lipopolysaccharide, that enter the liver sinusoids.

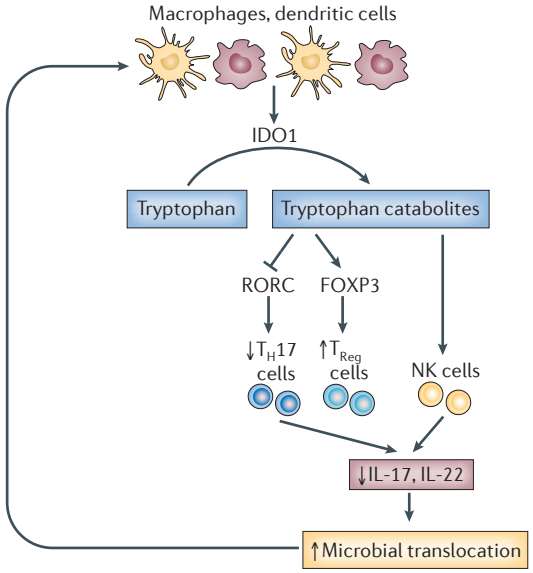


Figure 2 | **Indoleamine 2,3-dioxygenase 1 pathway.** Interferons and Toll-like receptor (TLR) ligands drive the production of indoleamine 2,3-dioxygenase 1 (IDO1) by macrophages and dendritic cells[94]. IDO1 converts tryptophan to tryptophan catabolites, such as kynurenine. These tryptophan catabolites simultaneously induce the expression of forkhead box protein P3 (FOXP3; which promotes regulatory T cell differentiation) and block the expression of RAR-related orphan receptor C (RORC; which promotes T helper 17 (T_H17) cell differentiation), leading to decreased production of T_H17 cells and increased production of regulatory T (T_{Reg}) cells. In addition, these tryptophan catabolites drive natural killer (NK) cells to produce less intereukin-17 (IL-17) and IL-22, compounding the effect of the decreased IL-17 and IL-22 production that is due to loss of T_H17 cells from the intestine. Thus, there are fewer T_H17 cells to control microbial growth and less IL-22 to induce mucosal healing, and this potentiates microbial translocation and provides more TLR ligands to induce further IDO1 production.

the intestine and allow them to enter the circulation. In addition, defective phagocytosis of microbial products by the few macrophages present may contribute to impaired clearance of these products[20,107].

Consequences of microbial translocation

Microbial translocation has been suggested to be a driver of systemic immune activation, which is associated with numerous HIV-associated pathologies.

Role in immune activation. HIV-infected individuals show increased inflammation and immune activation compared with uninfected controls. In response to LPS administration, they produce more pro-inflammatory TNF, IL-6 and IL-8 and less anti-inflammatory IL-10 and IL-1 receptor antagonist[108]. Moreover, HIV-infected individuals produce increased levels of sCD14 (REFS 22,35), sCD163 (REF. 28) (which is shed by activated monocytes and macrophages), TNF[35] and IFNα[22] compared with uninfected individuals. In fact, IFNα was found to colocalize with *E. coli* proteins in the lamina propria and axillary and mesenteric lymph nodes[20], suggesting a direct association between type I IFNs and microbial translocation. Similarly, in the absence of HIV infection, individuals with hepatitis B or hepatitis C infection and cirrhosis have increased *E. coli* protein deposition and increased CD14 density in the liver[102], suggesting a common pathway in which monocyte activation by LPS is associated with fibrosis. One possible explanation for this increased production of pro-inflammatory mediators in HIV infection is that HIV or HIV-derived ligands sensitize monocytes to TLR4 stimulation by stimulating TLR8, leading to heightened responses of monocytes to LPS[36,109].

These pro-inflammatory cytokines are only one potential contributor to increased T cell activation. Increased LPS levels also correlate with an increased frequency of $CD8^+$ T cells expressing markers of activation in untreated individuals with chronic HIV infection or AIDS[30] and among HIV controllers[23] (individuals who control the virus and have undetectable HIV RNA levels despite not taking ART). Among HIV controllers, a 10% increase in the frequency of activated $CD8^+$ T cells was associated with 101 fewer $CD4^+$ T cells per mm^3, suggesting a link among microbial translocation, increased $CD8^+$ T cell activation and greater $CD4^+$ T cell depletion. In addition, both *in vitro* and *in vivo* experiments have shown that LPS can increase the percentage of monocytes and $CD4^+$ T cells that express CCR5, the HIV co-receptor, suggesting that microbial translocation may increase the number of target cells for HIV[110–112].

Consistent with these data, increased concentrations of plasma 16S rDNA are associated with higher frequencies of activated $CD8^+$ T cells among subjects taking ART[15]. Ugandan individuals with untreated chronic HIV infection and high levels of sCD14, reflecting LPS-induced monocyte activation, had a higher frequency of activated and terminally differentiated $CD4^+$ T cells (as measured by the expression of the activation markers CD38, HLA-DR and the differentiation marker programmed cell death protein 1 (PD1))[47]. High sCD14 levels also correlate with a high frequency of activated $CD4^+$ T cells among virologically suppressed subjects on ART, suggesting a link between microbial translocation-induced monocyte activation and $CD4^+$ T cell activation[113].

In addition to promoting T cell activation, high LPS levels correlate with enhanced entry into the cell cycle, as indicated by expression of Ki67 (REF. 114). Increased frequencies of $CD4^+$ T cells expressing Ki67 in the peripheral blood, colon and ileum, and $CD8^+$ T cells expressing Ki67 in the ileum correlate with high LPS levels in HIV-infected subjects[39]. An increased frequency of $Ki67^+$ $CD4^+$ T cells in the peripheral blood has been associated with increased $CD4^+$ T cell depletion[86]. This may be because $CD4^+$ T cell depletion allows increased microbial translocation, resulting in increased pro-inflammatory cytokine production and subsequently cellular activation and cell cycle entry. It is also possible that the lack of total $CD4^+$ T cells leads to homeostatic $CD4^+$ T cell proliferation. However, such substantial proliferation requires enteric organisms, as it does

not occur in germ-free mouse models, indicating that microbial products play a crucial part in such homeostatic proliferation[115].

Association with CD4+ T cell depletion and HIV RNA levels. HIV-infected individuals with lower CD4+ T cell counts, regardless of whether they are taking ART, have higher plasma levels of LPS[30,116,117] and sCD14 (REFS 47,113,118). CD4+ T cell depletion in this context has been associated with high viral loads, which correlated with higher LPS levels in a study of both treated and untreated HIV-infected subjects[30,34,119]. Furthermore, higher sCD14 levels correlate with higher HIV RNA levels[47,118,120].

Compared with immunologic responders, immunologic non-responders have higher LPS levels (which are associated with an increased frequency of activated CD4+ and CD8+ T cells)[121], higher sCD14 levels (which are associated with increased numbers of activated CD4+ T cells and decreased nadir CD4+ T cell counts)[9,122] and detectable 16S rDNA[121]. Increased immune activation[9,10,123] and low nadir CD4+ T cell counts[9,122] both correlate with impaired recovery of CD4+ T cell counts in patients on ART, and higher LPS levels are associated with lower CD4+ T cell counts after ART interruption[119]. A reduction in circulating CD4+ T cells may impair GALT restoration by decreasing the number of T cells that could traffic to the intestine[37], leading to bacterial overgrowth and increased microbial translocation[124]. Thus, there is a tight interplay among low nadir CD4+ T cell counts, microbial translocation, immune activation and immunologic non-response.

One potential mechanism by which microbial translocation may impair CD4+ T cell recovery in patients on ART is by causing lymphatic tissue fibrosis, which can be quantified by visualizing collagen deposition[125]. In chronic SIV infection, LPS can be detected in the mesenteric and axillary lymph nodes (primarily in the T cell zone) and in the colon[20]. Similarly, in patients with chronic HIV infection on ART, a greater density of collagen I deposition in mesenteric and axillary lymph nodes and GALT is associated with a smaller CD4+ T cell population in the lymphatic tissue and with reduced CD4+ T cell recovery[125,126]. This collagen deposition is probably driven by transforming growth factor β1 (TGFβ1), which is produced in response to local immune activation induced by LPS[127,128]. Indeed, the degree of fibrosis in the GALT correlates directly with CD4+ T cell activation and inversely with CD4+ T cell count[129]. Collagen deposition in the T cell zone is associated with destruction of the fibroblastic reticular cell network, resulting in loss of access of T cells to IL-7, which naive CD4+ T cells require for survival, and obstruction of antigen presentation by dendritic cells to T cells[130]. Thus, microbial translocation drives the immune activation that causes lymphatic tissue fibrosis and thereby impairs CD4+ T cell recovery.

Association with clinical outcomes. Even when on ART, HIV-infected individuals are at increased risk of developing non-infectious complications, which are associated with enhanced immune activation and inflammation. For example, HIV-infected individuals are at increased risk for neurocognitive impairment. Individuals with HIV-associated dementia have higher LPS, LBP and sCD14 levels and lower EndoCAb levels than HIV-infected subjects without detectable cognitive disorders[33,131]. Increased sCD14 levels have also been observed in subjects on ART with cognitive impairment and brain atrophy, potentially as a result of microglial activation or monocyte infiltration in the brain, which leads to increased production of neurotoxins (such as arachidonic acid) and the pro-inflammatory cytokines TNF and IL-1β[132,133]. Thus, LPS-induced monocyte activation is associated with neurocognitive dysfunction.

HIV infection is also associated with an increased risk of developing cardiovascular disorders. HIV-infected individuals have a twofold increased risk of myocardial infarction[134] and an increased risk of arterial and venous thromboses[135,136]. Furthermore, in HIV-infected subjects, increased levels of D-dimer (a marker of activation of the coagulation cascade and fibrin formation and lysis) are associated with increased mortality[7]. Cardiovascular disease may be a consequence of increased levels of microbial products. LPS and flagellin have both been shown to increase the expression of tissue factor (TF), which initiates the coagulation cascade on the surface of monocytes[137]. Indeed, the frequency of monocytes expressing TF is increased in HIV-infected individuals and correlated with levels of sCD14, D-dimer and activated CD8+ T cells[137]. Similarly, HIV-infected individuals have a higher frequency of activated platelets and TF-expressing platelets, which correlates with the frequency of TF-expressing monocytes and with circulating sCD14 levels[49]. These findings suggest that monocytes and platelets produce TF, which is known to contribute to arterial plaque development, in response to the increased LPS levels in HIV-infected individuals[138,139]. TF initiates the coagulation cascade, leading to the production of fibrin, which is degraded into D-dimers. Consistent with this, HIV-infected individuals with increased arterial plaque deposition have higher sCD14 levels than those with normal carotid intima media thickness[140]. Thus, increased microbial translocation in HIV-infected individuals may contribute to the hypertensive and hypercoagulable state observed in these individuals.

Recently, increased microbial translocation has been associated with progression of disease and mortality in HIV infection. In a study of individuals on zidovudine monotherapy, those with more severe disease (based on CDC criteria) had higher serum sCD14 levels, with the highest levels observed in subjects with AIDS[45]. Among individuals not on ART, higher LPS and sCD14 levels at baseline predicted faster progression towards CD4+ T cell counts of less than 200 cells per mm^3, AIDS manifestation and death, although only LPS was significantly predictive in a multivariate analysis[118]. Among subjects enrolled in the Strategies for Management of Antiretroviral Therapy (SMART) study, most of whom were on ART at enrolment, high baseline sCD14 levels predicted all-cause mortality (death from any cause, including infection, cardiovascular event, malignancy and other aetiologies)

Nadir CD4+ T cell counts
An individual's nadir CD4+ T cell count is their lowest recorded CD4+ T cell count since their diagnosis with HIV infection.

T cell zone
The region of lymphatic tissue where most CD4+ T cells reside and interact with antigen-presenting cells, growth factors and cytokines.

Carotid intima media thickness
A measurement of the thickness of the two inner layers of the carotid artery, often performed by ultrasound, that is used to quantify atherosclerotic disease.

independently of CD4+ T cell count, HIV RNA levels and levels of other pro-inflammatory markers[8]. In this study, fewer than 10% of deaths were due to opportunistic infections; the most common causes of death were cardiovascular events, malignancy and unknown (which were presumed to be sudden cardiac arrests[141,142]), all of which are associated with inflammation. Indeed, mortality has been associated with both higher sCD14 levels and faster increases in sCD14 levels, reflecting the rapid onset of severe inflammation[120]. The fact that numerous studies associate clinical end points with sCD14 and only one with LPS may reflect not only the difficulties in measuring LPS but also differences in LPS clearance mechanisms, genetic factors that determine monocyte-responsiveness to LPS and the differential response of LPS and sCD14 following ART initiation. The significance of LPS-induced monocyte activation in the setting of malignancy and other end points needs further investigation.

Therapeutic options

Most therapeutic studies of HIV-infected individuals have focused on suppressing the virus, but recently approaches that target the microbial products and their downstream effects have been evaluated.

Effects of ART. Numerous cross-sectional studies have evaluated the effects of ART on microbial translocation, but the data are conflicting. Some studies demonstrated lower LPS levels among those on ART[35], some demonstrated lower sCD14 levels[21,32] and some showed neither[8,39].

However, the data from longitudinal studies are more consistent. In several cohorts of HIV-infected subjects, both adults and children have shown decreased levels of LPS[22,29] and sCD14 (REFS 27,29) after the initiation of ART, but these levels were still higher than those in uninfected individuals[22,27,29]. In a longitudinal study of ART-naive subjects placed on treatment with raltegravir, tenofovir and emtricitabine, sCD14 levels fell significantly after 2 days of ART, and LPS levels fell significantly within 8 weeks of ART initiation[51]. The decline in LPS levels in this setting may depend on the recovery of LPS clearance mechanisms, including increased clearance by Kupffer cells and neutralization by endotoxin antibodies, as suggested by a treatment-interruption study that showed an inverse relationship between the levels of LPS and EndoCAb after resuming ART[24].

ART may in fact lead to some degree of repair of the gastrointestinal tract. Initiation of ART during primary HIV infection results in almost complete reconstitution of intestinal CD4+ T cells, but initiation of ART in chronic HIV infection leads to incomplete restoration of intestinal CD4+ T cell counts[89,126,143,144]. Microbial translocation may decrease upon the initiation of ART owing to partial T_H17 cell restoration and improved LPS clearance mechanisms. In addition to increasing EndoCAb levels (as noted above), ART may increase the numbers of Kupffer cells, as shown by data from individuals with HIV and hepatitis C virus co-infection[101]. Thus, ART can facilitate partial intestinal healing and decrease microbial translocation, but not to the level observed in HIV-uninfected individuals.

Therapeutic opportunities. Novel approaches targeting the microbial products or their downstream effects are being evaluated to attenuate the clinical consequences of heightened immune activation. Several agents may be able to decrease circulating LPS levels (FIG. 3). Sevelamer — which is an orally administered, non-absorbable polymer that is used to treat hyperphosphataemia in individuals on haemodialysis — was found to bind LPS and decrease LPS-mediated activation of monocytes *in vitro*[145]. *In vivo*, sevelamer decreased LPS levels by 80% and CRP levels by 78% in subjects on haemodialysis[146]. Rifaximin, an orally administered antibiotic with minimal systemic absorption, targets Gram-negative, Gram-positive and anaerobic bacteria, and has been found to decrease LPS levels in both systemic and hepatic circulations by as much as 50% in subjects with alcoholic cirrhosis after 4 weeks of treatment; levels of the pro-inflammatory cytokines IL-1, IL-6 and TNF also decreased[147,148]. Recently, pigtail macaques given both rifaximin and sulphasalazine simultaneously with SIV challenge were reported to have lower plasma levels of sCD14 and pro-inflammatory cytokines, less T cell activation and decreased CD4+ T cell decline, although it is unclear whether these effects are a result of rifaximin-induced gut microbiome alteration or sulphasalazine-induced intestinal healing, or both[149]. Hyperimmune bovine colostrum, which contains LPS-specific immunoglobulin, had been shown to decrease LPS levels in patients who have undergone surgery, but it showed no effect on LPS or sCD14 levels in HIV-infected individuals on ART[50]. The composite results of these studies are promising and suggest that further investigation of LPS-lowering medications in the setting of HIV infection is needed.

Recently, a small study of ART-naive HIV-infected individuals showed that prebiotic supplementation with an oligosaccharide mixture increased the percentage of bifidobacteria (a commensal bacterium that lacks LPS, has anti-inflammatory properties and normalizes circulating LPS levels in a mouse model of endotoxaemia[150]) in the gut microbiota[151]. This supplementation also decreased sCD14 levels[151]. Given the role of commensals in protecting against intestinal damage[152], this may prove to be another viable intervention, as it may lower the translocation of not only LPS but also other microbial products, such as flagellin and peptidoglycan.

By blocking NF-κB, chloroquine and hydroxychloroquine inhibit signalling through several TLRs, including TLR4 and TLR9 (REFS 153,154). Both chloroquine treatment of HIV-infected subjects not on ART and hydroxychloroquine treatment of those on ART led to a decrease in activated CD4+ T cells and LPS levels. Chloroquine promoted a decrease in activated CD8+ T cells[153], and hydroxychloroquine induced a decrease in IFNα-secreting plasmacytoid dendritic cells, IL-6 and TNF, and an increase in the percentage of circulating CD4+ T cells[154], suggesting that decreasing microbial translocation-mediated immune activation may improve CD4+ T cell recovery.

Prebiotic
Prebiotics are substrates that are preferentially metabolized by a single probiotic genus or species and may thus be used as dietary supplements to promote targeted growth of these microorganisms.

Plasmacytoid dendritic cells
Immature dendritic cells with a plasmacytoid morphology. They produce type I interferons in response to viral infection.

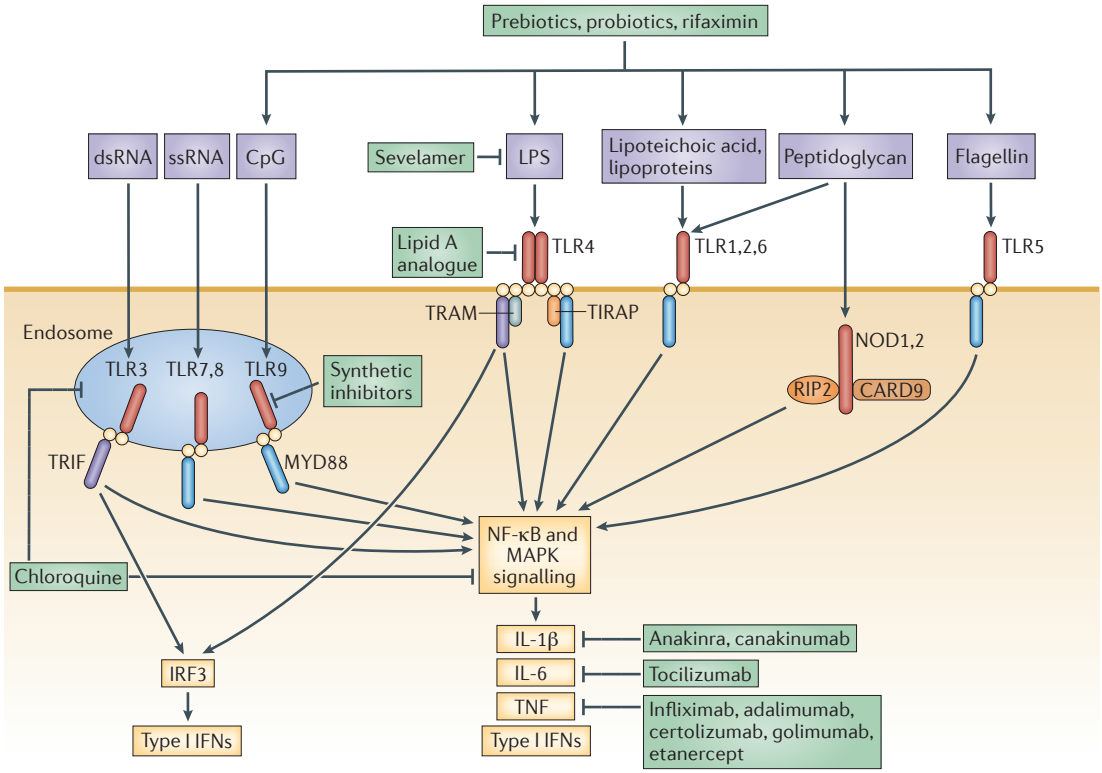


Figure 3 | **Therapeutic interventions to attenuate microbial translocation-induced immune activation.** Numerous agents exist that may interfere with microbial signalling (see BOX 1 for details of the signalling pathways). Sevelamer targets lipopolysaccharide (LPS)[145], and prebiotics, probiotics and rifaximin work by altering the intestinal flora and potentially inducing epithelial healing[148,151]. Thus, these agents may reduce signalling by numerous Toll-like receptor (TLR) ligands as well as peptidoglycan. A lipid A analogue that binds TLR4 has been used in clinical trials of sepsis, and synthetic inhibitors that block TLR9 are currently being developed. Chloroquine blocks signalling through TLR3, TLR7, TLR8 and TLR9 and thereby interferes with bacterial DNA signalling, but it also may act further downstream in blocking nuclear factor-κB (NF-κB)[153,154]. Even further downstream, monoclonal antibodies directed towards the pro-inflammatory cytokines produced upon NF-κB activation may attenuate immune activation. CARD9, caspase recruitment domain-containing protein 9; dsRNA, double-stranded RNA; IFN, interferon; IL, interleukin; IRF3, IFN regulatory factor 3; MAPK, mitogen-activated protein kinase; MYD88, myeloid differentiation primary-response protein 88; NOD, nucleotide-binding oligomerization domain; ssRNA, single-stranded RNA; TIRAP, Toll/IL-1R domain-containing adaptor protein; TNF, tumour necrosis factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-domain-containing adaptor protein inducing IFNβ.

Other TLR antagonists may also attenuate immune activation. Eritoran, a lipid A analogue that binds TLR4, has been shown to block LPS activity in healthy volunteers, triggering reduced CRP, TNF and IL-6 levels[155], although a study of eritoran to treat sepsis did not show a significant benefit[156]. TLR9 antagonists may also decrease immune activation by blocking CpG-mediated signalling[157].

In addition, monoclonal antibodies directed against cytokines and cytokine signalling interfere downstream of microbial product signalling and could thereby reduce immune activation driven by microbial translocation. Anakinra and canakinumab are indicated for rheumatoid arthritis and cryopyrin-associated periodic syndromes, respectively, and both interfere with IL-1 signalling. Tocilizumab is an antibody specific for the IL-6 receptor and is used to treat individuals with rheumatoid arthritis that is refractory to TNF inhibitors. A monoclonal antibody against IFNα is currently being investigated for the treatment of systemic lupus erythematosus. As increased IFNα production is one of the sequelae of LPS-induced immune activation, and IFNα may contribute to both lymphatic tissue fibrosis and increased IDO1 expression, an IFNα antibody may have pleiotropic beneficial effects in HIV-infected individuals. Thus, the discovery and exploration of the role of microbial translocation and its downstream effects on HIV disease progression have inspired research that aims to block this process and ultimately augment the benefits of ART.

Conclusion

Microbial translocation is increased in individuals infected with HIV. Increased levels of circulating microbial products are strongly associated with increased immune activation, and the host response to those products predicts clinical outcomes, including immunologic non-response and death.

Despite these strong associations, it has not yet been conclusively demonstrated that microbial translocation causes immune activation rather than merely occurring

concomitantly with immune activation. It remains unclear whether LPS is the primary instigator of immune activation or whether other microbial products, such as flagellin and peptidoglycan, also have prominent roles. In addition, it has not yet been shown that decreasing microbial translocation will decrease immune activation and improve clinical outcomes. Studies aimed at interfering with microbial translocation and its subsequent immune activation will clarify whether increased microbial translocation drives immune activation and mortality. They should also illuminate therapeutic options to improve outcomes for HIV-infected individuals.

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Acknowledgements
We thank the members of the Cleveland Immunopathogenesis Consortium (BBC), funded by US National Institutes of Health grant AI-76174, for stimulating discussions.

Competing interests statement
The authors declare no competing financial interests.