MICROBIAL TRANSLOCATION AND ITS CLINICAL SIGNIFICANCE

RESEARCH ARTICLE

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Citation style for this article: Kaonga P, Kaimoyo E, Kelly P. Microbial translocation and its clinical significance, Health Press Zambia Bull. 2018 2(4); pp 5-16.

The literature was searched in PubMed Medline National Library of Medicine from 1990 to 2016 were used. The following words were used: 'microbial translocation' and 'clinical significance,' or 'biomarkers,' or 'toll-like receptor,' or 'pathogen-associated molecular pattern.' We found 3,300 published manuscripts using the above search. Of 3,300 manuscripts, we dropped 2087 and 723 manuscripts either they did not suit this review or were not in English; 490 manuscripts were selected for this review. From the literature, there is evidence that microbial translocation occurs in both animals and humans, but unlike in animals, its clinical significance remains questionable in humans. This could partly be explained by the current lack of a single acceptable sensitive and accurate biomarker to detect microbial translocation. Additionally, the extent to which microbial translocation in animals can be demonstrated cannot apply to humans for the sake of research without an underlying disease. In humans microbial translocation is associated with many conditions and microbial products may lead to systemic inflammation and immune activation. Although some of the microbial products or Pathogen-Associated Molecular Patterns (PAMPs) have been studied, their clinical importance is not well established, and the assays developed to measure PAMPs in blood have not been developed or validated for clinical use. However, a few molecules of microbial origin have been used as biomarkers of microbial translocation in many disease conditions. The innate immune system detects all PAMPs through cells such as macrophages, dendritic cells, and monocytes. Detection of PAMPs through pathogen recognition receptors such as Toll-like receptors which result in the activation of the transcription factors, NK-κB, resulting in the production of pro-inflammatory cytokines. We provide a synthesis of the current understanding of the nature of microbial translocation, PAMP-receptor interaction and the health significance of microbial translocation in humans.

Introduction

The human intestinal epithelial layer is one cell thick and plays two major roles. It absorbs the much needed nutrients and water for the host and excludes bacteria, antigens, and other non-self substances from crossing the intestinal barrier into sterile sites. The human gut contains a variety of bacteria species mainly the non-pathogenic ones as part of its normal microbial flora. Under normal circumstances, an individual's gut microbiome contains around 105 colony forming units (CFU)/ml of non-pathogenic bacteria in the jejunum, around 108 CFU/ml in the distal ileum and cecum and up to 1012 CFU/ml in the colon [1]. Examples of insignificant intestinal bacteria include members that belong to Actinobacteria and Proteobacteria while the majority of intestinal bacteria are members of phylogenetic lineages, Firmicutes and Bacteroidetes [2]. It has been estimated that up to 1,000 microbial species belonging to Firmicutes or Bacteroidetes species are uncultivable [3] which makes difficult to identify them by routine laboratory methods. To date, the exact relationship between human gut and microbiota has not been fully elucidated. Even less research has been conducted to understand the immune response to gut microbiota in humans.

When the bacteria count in the upper jejunal aspirate is greater than 105 CFU/ml or if the presence of colonic bacteria is detected, the condition is referred to as small intestinal bacterial overgrowth (SIBO) [4]. In such cases, aerobes tend to be fewer than anaerobes in the ratio of about 1:100 [5]. Intriguingly, it is the Gram-negative bacteria of the less abundant of the two groups of bacteria that are mainly involved in microbial translocation (MT) even across normal histological intestinal epithelium [6]. Here, we discuss MT, the underlying mechanism, and some associated conditions and its clinical significan**ce.**

Exclusion of microorganisms by mucosal barrier

The intestinal mucosa is mainly composed of muscularis mucosae, lamina propria and epithelium [7]. It is monitored by immune cells mainly of the innate immune system such as dendritic cells, mast cells, and macrophages together with the lymphoid system. In health, the anatomical structure of the intestinal surface is covered by

mucins secreted by goblet cells and gastric mucus which forms a gel-forming barrier. These exclude external elements and the majority of bacteria from direct contact with cells of mucosal layer. The mucosal layer components consist of water, electrolytes, phospholipids, proteins, and phospholipids [8]. Also, the ability of the microbes to cross the barrier is hampered by antimicrobial agents such as the defensins HD5 and HD6, as well as a large amount of IgA [8]. The epithelial layer is composed of many other cells and biological molecules. These include enterocytes involved in absorption and hormone production. They do not live for a longer time and are regularly replaced within few days. Also, paneth cells are also found in the crypts. These are involved in the production of growth hormones, digestive enzymes and defensins [9]. The gut barrier is also composed of a gastric barrier acid which kills many ingested bacterial and viral pathogens. The epithelial layer also possesses two essential components; the villi and the crypt, which are significant in the absorption of nutrients, secretion of fluids containing electrolytes and serve some immune functions [10, 11]. The villi are finger-like protrusions that increase the surface area for absorption and the crypt are at the bottom of villi and secrete essential fluids containing antimicrobial peptides. Altogether these different elements form an intestinal mucosa with a barrier, immune and absorptive functions [12].

The enterocytes are joined to the adjacent cell by a complex of tight junction proteins which are composed of claudin proteins and play a significant role in the selective regulation of ionic solutes passing through the cell. In case the intestinal barrier is compromised resulting in marked loss of barrier function, microbes or their products cross the barrier through the paracellular pathway [13]. The paracellular pathway is more permeable than the transcellular pathway which involves passive passage of substances through the space between adjacent cells. The transcellular pathway involves the action of specific channels, which move materials passively or actively across cell membranes [10]. Evidence suggests that these two routes are possibly controlled autonomously [14, 15]. The paracellular route is referred to as the leaky pathway because it allows bigger particles to transverse through such as microbial components which are unable to cross through the cells. Tight junction proteins include the family of 18 claudins, which play a significant role in the determination of pole charge. Other proteins which play important roles in the epithelial barrier function include desmosomes and adherens which form junctions joined to the actin cytoskeleton [10, 16]. Some in-vivo and in-vitro studies have demonstrated that lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α) increase permeability of the gut thereby exacerbating the microbial translocation

Assessment of intestinal integrity

It is important to assess the integrity of the intestinal barrier which can be achieved by staining the intestinal biopsy with hematoxylin and eosin (H&E) and reveal any alterations of the mucosa [18]. This is of particular importance in that the composition and integrity of tight junction proteins such as occludins and claudins, as well as zonula occludens, can be analyzed [19]. These proteins can also be studied in detail by immunofluorescence staining technique using specific antibodies that bind specifically to the proteins under investigation [20]. Some tight junction proteins such as claudin 4 have also been evaluated using Western blotting [18] or by quantitative polymerse chain reaction (qPCR). There are many more proteins also involved in maintaining the integrity of the tight junctions and because of the complexity of their interactions in-vivo it may not be possible to single out the single most important protein involved in the maintenance of barrier integrity. One of the most promising developments in understanding the histology of the mucosa in-vivo is the use of confocal laser endomicroscopy, which is capable of revealing small gap within the epithelial layer and has already been used in individuals with environmental enteropathy .

Clinical significance of microbial translocation

There is overwhelming literature on experimental studies that have demonstrated that MT occurs in animal models under various conditions. These include environmental enteropathy (EE) [21], colitis [22], liver cirrhosis [6], small bowel obstruction and ischemia in vivo [23], hemorrhagic shock [24], trauma [25] as well as due to abuse of opiates such as morphine [26]and acute pancreatitis [27]. However, in humans, the clinical significance of MT remains to be elucidated, and some conditions where some studies have been done are discussed here.

Microbial translocation in environmental enteropathy

It has been suspected for a long time that people living in areas of poor sanitation and hygiene especially those common in tropical countries, are affected by a widespread phenomenon of asymptomatic abnormal structural and functional changes of the small intestine referred to as EE [28]. No single specific agent is responsible for EE, but it is hypothesized due to repeated exposure to fecal-oral contamination. It is associated with some factors including reduced responses to oral vaccines [29, 30], micronutrient deficiencies, growth failure and stunting in children [31] and MT [18, 32]. This subclinical condition is characterized by loss of intestinal barrier function, chronic intestinal inflammation,

microbial translocation and chronic immune activation [28, 33, 34]. Due to compromised gut barrier, microbes and their products translocate from the gut into systemic circulation resulting in immune activation. Chronic immune activation may lead to microcirculatory dysfunction, intravascular coagulation and hemodynamic disturbances leading to hypotension, metabolic derangements septic shock and death [25].

Association between fetal deaths and socio-economic and demographic variables

The chi-square results in table 1 with a p-value less than 0.05 at 95% confidence interval (CI) indicate that there was a statistically significant relationship between each of the following independent variables and the dependent variable (fetal deaths); age of mother, years lived in a place of residence, children ever born, number of living children, marital status, fertility preference, person who makes decisions on the mothers health care. The percentage of women with fetal deaths increased with increasing age; more women in rural areas (5.5%) had fetal deaths compared to urban women (5.1%); 8.3% of women who lived in a place of residence less than a year had a fetal death; women with a higher education had a fetal death (6%); the percentage of fetal deaths reduced with increase in the number of children ever born and the number of children alive; 5.8% of fetal deaths were among women in a union; 6.5% of fetal deaths were among women who were undecided about fertility preference (undecided about having another child); and 11.4% of fetal deaths occurred to women's whose health care was determined by someone else. However, women's socio-economic characteristics such as; region, education status, religion and wealth index were not significantly associated with fetal deaths.

Microbial translocation in HIV infection

HIV is known to infect the lymphocytes and macrophages of the intestinal mucosa [3]. In severely immunosuppressed people with HIV infection, particularly those with full-blown AIDS, chronic diarrhea, and weight loss are common [37]. In such individuals, the gut appearance at the microscopic level is comparable to that of EE in particular in the late stage of the disease [35]. The intestinal epithelium may be damaged by HIV allowing the pathogens within the gut to cause more damage to the gut due to host immune suppression culminating into enteropathy which may promote HIV disease [38]. Some in-vitro experiments have reported that the HIV glycoprotein gp120 interrupts the tight junction proteins [39] and also the actin cytoskeleton and microtubules are modified by the HIV transactivator factor Tat resulting in apoptosis, although HIV does not infect enterocytes, the mechanism is unclear. The suggested effects on the gut mucosa in HIV patients including paracellular permeability has been demonstrated by using immunohistochemistry techniques [40]. Taken together, these increase the chances of microorganisms in the gut to move across the intestinal barrier.

Microbial translocation in Patients with Cirrhosis

MT has been reported to occur in about 30% in patients with cirrhosis [41] and has been demonstrated to be even as high as 78% in mouse models. Bellot [40] and others showed that 16S rRNA, a biomarker of bacterial translocation, was elevated in patients with cirrhosis. This has been used as a biomarker of MT by others but with no correlation with the severity of cirrhosis. Another study demonstrated that cirrhotic patients had elevated lipopolysaccharide-binding protein (LBP) compared with healthy controls and concluded that there was a possible involvement of bacteria and their products. The conditions were improved by antibiotics [42]. Others have demonstrated MT from the positive bacteriological culture from surgical removal of mesenteric lymph nodes (MLNs) in animal models [43]. The pathogenesis of MT in cirrhosis may manifest in different ways, but the most suggested include small intestinal bacterial overgrowth (SIBO) which most investigator define as 105 CFU/ml of proximal jejunal aspirate [44]. However, the main challenge for diagnosis of SIBO procedure using proximal jejunal aspirate is the invasiveness of the procedure. For this reason, the non-invasive method of the hydrogen and methane breath tests after an oral dose of glucose or lactulose are preferred [45]. MT was only present is about 50% of cirrhotic with SIBO mice suggesting that other factors apart from SIBO were responsible for MT in mice. Some investigators have reported structural and functional alterations in cirrhosis which predisposes mice to microbial translocation [46]. The general immunological impairment of the intestinal immune system has been shown to promote MT in cirrhosis. Taken together, these results may partly explain why MT in patients with cirrhosis is common.

Microbial translocation in Inflammatory Bowel Diseases

Although the exact cause of inflammatory bowel diseases (IBDs) such as Crohn's disease (CD) and ulcerative colitis (UC) is still elusive, it is hypothesized that some individuals are genetically predisposed to have the diseases especially those with abnormal immune response to intestinal microbiota [47]. Studies have shown that patients with CD and UC have higher levels of plasma and tissue 16S rRNA compared with healthy controls suggesting MT [48]. The levels of 16S rRNA are also higher in patients with active disease than in those with the inactive disease but no difference regarding transcription factor, Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), which plays a key role in the regulation of the response. The 16S rRNA levels and the NOD2 transcription factor have reported not to be associated with MT [49]. On the contrary, different studies showed that 16S rRNA in the serum of IBD patients was related to NOD2. The results were further supported by elevated levels of pro-inflammatory cytokines [50, 51]. Some studies have reported on the

type of bacteria phylum that is implicated in IBDs. Some studies have demonstrated that Bacteroidetes species are more commonly found in intestinal biopsies of CD patients compared with those with irritable bowel syndrome or healthy controls. The studies have noted that significant increase in both CD and UC whether active or inactive compared with healthy controls may be linked to some factors including smoking, diet, and biopsy or stool sample. Variations in the activity of the disease may be explained by other factors including sample size [52, 53].

Other conditions in which microbial translocation is of clinicalsignificance

In industrialized countries, one of the causes of liver disease is the non-alcoholic fatty liver disease (NAFLD). This mostly happens when there are insulin resistance and obesity compounded with steatohepatitis which may originate from simple steatosis. Some studies have reported that obesity is associated with alteration in the gut microbiota [54] and increased intestinal permeability which may lead to MT [55, 56]. In one study [52], 94 neonates and infants who required parenteral nutrition due to gastrointestinal abnormalities successfully underwent surgical procedures. They were followed up for the development of septicemia due to MT in association with parenteral nutrition. Blood samples were cultured to diagnose MT samples from 15 patients were found to be positive for the condition with almost half of them associated with sepsis [57]. In 50 pediatric patients, who were immunosuppressed and were about to undergo small bowel transplant, the correlation between MT and acute rejection or preservation injury was evaluated. A positive culture from blood or liver biopsy was considered evidence of MT. In some cases, MT was associated with colon allograft, ischemia and acute rejection [58]. In monitoring postoperative sepsis in patients after undergoing laparotomy, patients were divided into those who had a positive bacterial culture in MLN and those who were negative. After a comparison had been made, patients who had a positive culture (MT) had more sepsis compared with those with negative culture (42.3% versus 19.9%) respectively [59]. In another study, at the time patients were undergoing general surgery, intestinal serosa and MLN samples were taken and cultured, and only 10% had MT with the occurrence of postoperative sepsis being two times more than in patients with MT but with similar mortality rate [60].

The diagnosis of MT by positive culture of a sample taken from MLN which is considered to a direct measure of MT by most investigators has several limitations. First, to get such a sample, surgery is needed. This is an invasive procedure which in itself would be too much in humans, unlike in animal model just to study MT only especially with its clinical significance in most conditions still unclear. Second, not all bacteria are cultivatable and so do not mean that negative growth the MLNs are free of bacteria. Third, culture is not very sensitive because a certain number of bacteria need to be present to get a positive result compared with methods like PCR. Fourth, even if samples of MLNs were to be obtained, it is not practical to get samples from all MLNs because if certain MLNs are found to be negative, it may not necessarily mean all MLNs do have bacteria. Fifth, some bacteria may be present in the non-viable state, and so they cannot grow on culture medium [61].

Is there a best specific biomarker of Microbial translocation?

The diagnosis of MT in humans, several optional methods have been proposed and tested with varying successes and limitations. These methods are based on various types of PAMPs including LPS, lipoteichoic acid, peptidoglycan (PDG) layer components and flagellin and their interactions with cell receptors. LPS a component of the Gram-negative bacteria have been detected in the plasma of humans with EE [18, 32], HSS [35] cirrhosis and HIV enteropathy [62] as a direct biomarker of MT since it's of bacterial origin. Other investigators have considered LPS as a surrogate biomarker of MT because of its short half life (2 – 3 hours) typically affected by some factors such as antibodies, immunogenetic and physiological variables [63]. In some conditions, detection of 16S rRNA in plasma of both human and animals has been used as a direct biomarker of MT. Real-time PCR does the detection and quantification. Studies have reported higher 16S rRNA copy number in HIV infected individuals compared with HIV negative individuals [62, 64, 65] while others found infected treatment naïve individuals had higher copy number compared with infected individuals who were on treatment. Others have also reported the presence of 16S rRNA in healthy individuals [66, 67] probably confirming what others have indicated that even in healthy individuals with intact epithelial barrier translocation takes place.

Peptidoglycan (PDG) layer is a component of both the Gram-positive and Gram-negative bacteria which is detected by Toll-like receptor 2 (TLR2) [68]. It makes about two-thirds of the Gram-positive bacteria cell wall and one-fifth of the Gram-negative cell wall. It has been detected in human plasma using silkworm larvae test. Although initial experiments were not in humans, its later use in some patients during the postoperative period of gastrointestinal surgery [69] revealed its potential for application in humans. The PDG was found to be higher in more than three-quarters of patients with severe bacterial infection [70]. Conversely, its use as a universal biomarker of microbial translocation has been difficult, and it is limited to surgical institutions. Flagellin is a subunit component of flagella present in motile bacteria [71], and an ELISA assay has been developed capable of detecting the protein. One of its first uses was in patients with short bowel syndrome who had either endotoxemia or without and in these patients marked increase in serum IgM, IgA, and IgG levels specific to flagellin were observed [72]. In patients

Table 1: Different PAMPs hypothesized to be involved in microbial translocation

PAMP	Origin	TLR	PAMP location	Adaptor protein	References
Lipopeptides	GPB	TLR1	Cell surface	MyD88	[79]
Peptidoglycan	GPB/GNB	TLR2	Cell surface	MyD88, Tram	
Double-stranded RNA					[82]
	GPB	TLR3	Endosome	TRIF	[81,82]
Flagellin	GPB/GNB	TLR4	Cell surface	MyD88	
Lipoteichoicacid, lipopeptides				TIRAP (Mal)	[83,84]
Single stranded RNA	RNA viruses	TLR5	Cell surface	MyD88	[85]
Single stranded RNA	GNB	TLR6	Cell surface	MyD88	
Unmethylated CpG- DNA /16S rRNA					[79]
Lipoteichoicacid, lipopeptides	GNB	TLR7	Endosome	MyD88	[85]
[79]	GPB/GNB	TLR8	Endosome	MyD88	[85]
Single stranded RNA	RNA viruses	TLR9	Endosome	MyD88	
Single stranded RNA					[84]
Unmethylated CpG- DNA /16S rRNA					

PAMP, Pathogen-Associated Molecular Pattern; GPB, Gram positive bacteria; GNB, Gram negative bacteria; TLR, toll-like receptor; MyD88, Myeloid differentiation primary response gene 88; TRAM, Toll- interleukin 1 receptor-domain-containing adapter-inducing interferon-β; TIRAP, Toll–interleukin 1 receptor domain–containing adapter protein; TRIF, Toll- interleukin 1 receptor -domain-containing adapter-inducing interferon-β

with CD, other investigators have reported presence of flagellin specific to Escherichia coli [73, 74] which was associated with compromised gut barrier. In the treatment of patients with HIV infection, anti-flagellin antibodies were used as biomarker of MT [75]. The use of this PAMP has been very narrow, and this warrants more studies to investigate its viability as a biomarker of MT in different diseases.

Lipoteichoic acid (LTA) is another PAMP that has been proposed as a diagnostic biomarker for MT. This is an equivalent of LPS in the Gram-negative bacteria, and it is shed during Gram-positive bacteria replication. It has been shown to induce the production of cytokines that are different from the ones observed when LPS stimulate transcription factors. If bacteria in the culture medium are exposed to antibiotics, LTA is secreted, and in human, its titers are reported to be higher in patients with chronic hepatitis C compared with healthy individuals [76]. Other studies have demonstrated that in patients with primary biliary cholangitis, LTA containing mononuclear cells were found in histological sections [77].

Other Biomarkers of Microbial Translocation

Indirect (surrogate) biomarkers are cellular biological molecules produced as a result of host immune response to MT. Biomarkers that have been reported to correlate with MT include sCD14 [31], LBP [35], intestinal-fatty acid binding protein [31, 78, 79] and EndoCab [79]. Other investigators have argued that sCD14 is not a biomarker of MT per se but rather a biomarker of monocyte activation but correlates well with LPS [80]. Another potential measure of direct biomarkers of MT is the use of plasma which stimulates a reporter cell line such as RAW-Blue mouse macrophages to express TLRs capable of detecting total PAMPs readout which are of microbial origin (Patrick Kaonga unpublished data). The total PAMPs activity can be measured by using detection reagent, QUANTI-Blue by spectrophotometer.

How immune system detects microbes and microbial products

After microbes and their components cross the intestinal barrier, innate immune system composed of unspecialized cells detect all non-self components from pathogen referred to as PAMPs through pathogen recognition receptors (PRRs) such as TLRs. TLR are germ-line encoded conserved receptors that are either expressed on the surface or endosomal that recognize various PAMPs [81]. PAMPs are essential for microbial survival and indispensable for microbial survival and fitness. Loss of patterns or even mutation can mean loss of life, so they have low major mutation rates. Second, they are not produced by host cells. Instead, they are produced by microorganisms, and this allows innate immunity to distinguish between non-self and self. Third, between microorganisms of a given class, PAMPs are invariant, meaning that not a lot of encoded PRRs are needed for microbial infection presence to be detected [82]. In humans, there are nine TLRs that have been described recognizing to interact with different PAMPs.

The main ligand for TLR1 is lipopeptide and for TLR6 are lipoteichoic acid and lipopeptides [81]. When these ligands are detected, they lead to secretion of pro-inflammatory cytokines by cells of the innate immune system including interferon-alpha (IFN-α), tumor necrosis factor-alpha (TNF-α) and interleukin-1-beta (IL-1β). Selected examples of PAMP-ligand interactions that have been studied are summarized (Table 1). TLR2 recognizes lipoteichoic acid and peptidoglycan which are major components of Gram-positive bacteria while TLR3 is only known to detect dsRNA [83, 84]. When LPS activates TLR4, its act together with membrane CD14 and Myeloid differentiation factor 2 (MD-2) proteins as co-receptors in recognition of LPS [85, 86]. Following recognition of these PAMPs, pro-inflammatory cytokines are produced which could worsen the inflammatory process and disease condition. Other receptors TLR5 recognize flagellin, TLR7 and TLR8 single stranded viral RNA in endosomal or lysosomal compartments [87] and TLR9 unmethylated CpG-DNA [86]. The feature of PAMPs detection by TLRs is followed by activation of transcription factor, NF-κB, leading to the production of pro-inflammatory cytokines.

Conclusions

From the literature reviewed, it is evident that MT in humans makes a significant contribution to several disease states, but its clinical significance is under studied. This is bound to change as more biomarkers become available. It is also likely to become possible to use these biomarkers to demonstrate the clinical significance of MT. Additionally, heightened and persistent immune activation which may originate from detection of PAMPs by TLRs which are expressed by immune cells and other organs may result in some clinical consequences.

Funding Statement: The work of the authors was supported by the Bill & Melinda Gates Foundation (Grant number OPP1066118)

Authors' contributions: Each author contributed sufficiently to the writing and revising the manuscript and approved for it to be published.

Conflict of interests: We declare that there is no conflict of interest.Nectur atquian daepedicia eumquatem int.

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