Review article

Microbiota milieu and mechanisms of intestinal Toll Like Receptors (TLRs) involved in chemotherapy induced mucositis

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ABSTRACT

Gut is not only of digestive but also of immunological importance because of the residing microbiota milieu. Pathological or certain therapeutic conditions may modify the normal commensal microflora. Mucositis, the most common untoward effect of chemotherapy, can also lead to this microbiotic imbalance. This shift leads to various molecular cascades which in turn trigger the action of Pattern Recognition Receptors (PRR's). Toll like receptor (TLR) is one such pattern recognition receptor. In the human body there are about 13 types of TLRs out of which TLR-2, TLR-4, TLR-5 and TLR-9 are intestinal specific. They respond through ligands such as bacterial derivatives like flagellin, Lipoteichoic acid, Lipopolysaccharides, microbial antigen or genetic material of the virus. In turn via adaptor molecules, TLRs alter the signalling mechanisms and further induce the activation of pro or anti-inflammatory cytokines based on the immunological need. Several of the studies have described the involvement of under twined mechanisms of TLRs during chemotherapy. Therefore, agonists and/or antagonists of these strategic molecules may play a key role in pathological and therapeutic aspects. Thus, this review is an attempt to focus on the involvement of TLRs and microbiota to different chemotherapeutic agents and thereby track the available mechanisms of functionality.

Keywords: Microbiota; Gut; Toll like receptors; Commensal and pathogenic bacteria; TLR-2; TLR-4; TLR-5; TLR-9.

INTRODUCTION

Mucositis is the commonest and unnerving complication of chemotherapy. Mucositis of GIT refers to the inflammation of the tract anywhere from the mouth to the anus. It is concerning because of the exertion of the patients to concentrate even on the basic requirements like food intake to immunological alterations. Nevertheless, GIT is the power plant of the microbiota. Innate and pathogenic bacteria involved thereby help in building immunity. It is the storehouse for numerous mechanisms involving immune responses. It's seen that various of these immune mechanisms are altered during mucositis. Sonis has shown that there will be extensive microbial infiltration during different stages of mucositis, which further triggers various molecular mechanisms (1). Different chemotherapeutic drugs showcase different ranges of mucositis. Present understanding proposes the series of intricate inflammatory events that could be initiated by chemotherapy-induced damage to the intestinal physiology (2). Other consequences including chemotherapy-induced diarrhoea has been associated with potential alterations in microbiota composition. These can be the contributing factors towards the dysregulation of the mucosal immune responses. Further, variation in gut microbiota may also influence the biochemical processes including the metabolism of xenobiotics (3).

These mechanisms are still obscure due to the diversity of the microbiota and immune components involved in it. One of the immunological tools of interest these days are TLRs, which are comparable to the police patrol. They identify the menaces and indicate response through the inflammatory changes. Besides, TLRs are activated by the components of the microbiota. Thus, in this review, an attempt to put together the various pathways of intestinal TLRs involving microbiota and their role in mucositis is made.

Microbiota and TLRs- A cross connection

Microbiota is an integral part of the gut homeostasis. It is reported that the human cells to microbial cells share a ratio of 1:1 (4). The endogenous microbiota of the GIT plays an important role in its physiology and pathology yet is not thoroughly characterized and is poorly defined. Significant functions of the normal microflora include epithelial cells protection, management of fat storage and stimulation of intestinal angiogenesis. The gut microbes not only influence the normal physiology such as drug metabolism and synthesis of vitamins, but also impact the pathological conditions including cancer (5).

Studies have reported the occurrence of dysbiosis due to chemotherapy and its influence on effectiveness of therapy (6). Among billions of microflora prevalent

ones include *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria* and *Verrucomicrobia.* Earlier study results show an increase in pathogenic microbes with reduced probiotic community during chemotherapy. reduction in number and type of microbiota mainly depends on the type of chemotherapeutic agent and the dosage. In healthy individuals, as well, due to the symbiotic relationship between the intestine and microbes, the gut mucosa has adapted extensively to the immunological properties. Thus, gut acts as an immunological organ due to the capacity of potentially antigenic microbes (7).

There are 2 types of bacteria present in the gut: commensal and pathogenic. Commensals are the native forms of microbiota like *Eubacterium lentum, Bifidobacterium species, Enterococcus faecium, Clostridium perfringes* which are innately responsible for intestinal homeostasis. Another form is the pathogenic, like *Salmonella typhi, Escherichia coli* etc., which are prevalent during pathological conditions (8). Whenever there is an imbalance in the ratio, (dysbiosis) these gut microbiota / probiota produce molecular patterns that decide the gut inflammatory response. There are 3 basic types of molecular patterns (9).

1. Commensal associated molecular pattern (CAMPs)
2. Pathogen associated molecular patterns and (PAMPs)
3. Damage mediated molecular patterns. (DAMPs).

While the former two are based on the number of microbial cells, the latter is based on the damage caused by any of the microbial cells (10). Nevertheless, all these patterns trigger the NFκB mediated pathway to show inflammation. The capacity of the microbiota to differentiate commensal from pathogenic cells play a key role and is brought about by the cells called as pattern recognition receptors (PRRs). The two main categories of PRRs are TLRs and Nucleotide binding oligomerization domains (NODs). TLRs are usually present on the surface of immunogenic and epithelial cells, whereas NODs are cytoplasmic (11).

Immunological pathways in the body, especially in the gut, show different shades of responses. TLRs are the receptors that are found throughout the body which respond to various stimuli called ligands. TLRs are transmembrane proteins which are present in endosomes and recognize gram-positive and gram-negative bacteria, flagellin, single and double stranded RNA, peptidoglycan, lipopolysaccharide (LPS), lipoteichoic acid and molecules from stressed or dying cells. TLRs are known to play a vital role in maintaining gut epithelial homeostasis(12). There are about 13 types of TLRs identified till now which are active in different tissues and cells among which, TLR-2, 4, 5 and 9 are associated with intestinal function. All these four TLR responses are microbiome dependent and recognize and respond to microbiota associated molecular patterns (MAMPs), in turn associated with DAMPs, CAMPs and PAMPs (13). There are a wide range of changes taking place in the activity and response of these TLRs during chemotherapy, as chemotherapy causes immense variation in the microbiota environment of the gut. Though the knowledge towards the lineage of TLRs is minimal, it has been shown that most of them are through mRNA expression in the intestine. Studies show that TLRs have shown varied responses to different chemotherapeutic agents. Thus, in this review we would focus on the involvement of TLRs and microbiota to different chemotherapeutic agents and thereby will track the available mechanisms of functionality of TLRs. Dose limiting chemotherapy targeting TLRs and microbiota would be beneficial in treating cancer with minimal mucotoxic effects (Fig. 1).

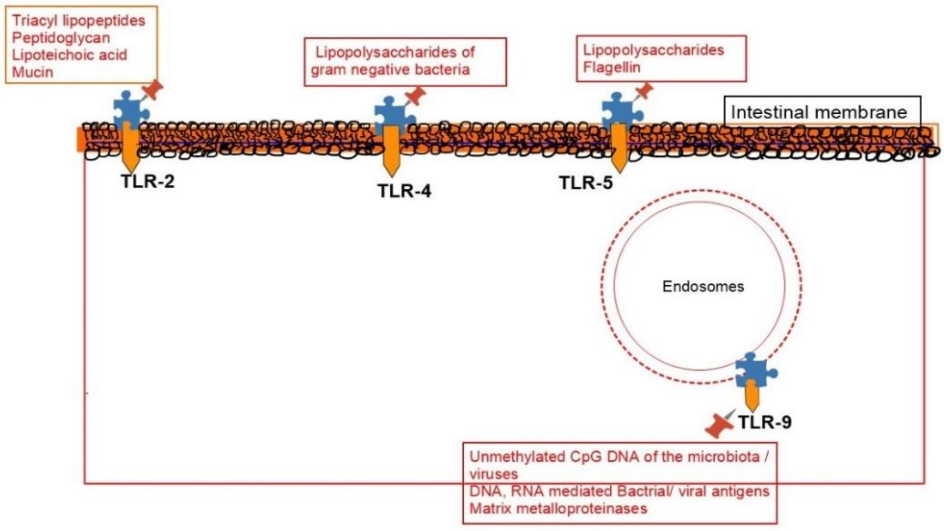

Fig. 1: The localization of intestinal TLRs with their ligands

TLR-2

TLR-2 is found to be vastly associated with chemotherapy induced mucositic models. This is attributed to its location, that it is subcellular. TLR-2 recognizes a variety of ligands from the microorganisms. It makes use of TLR-1 & TLR-6 to recognize ligands like bacterial acetylated lipopeptides, fungal pathogens, Lipoteichoic acid, Lipopolysaccharides, CD14 cells which help in bringing about the MyD88 protein dependent cascade (14). It also recognizes the injured or dead cells and makes its own adaptor proteins, few of them being MyD88, ATP binding cassette 1 (ABCB1), TRIP, TIRAP/MAL, SARM and TRAM. Different chemotherapeutic regimens react differently and activate / cause no response in the TLR-2 function. One of the studies showed that TLR-2 is highly concentrated in circulation in doxorubicin induced mucositis (15). Another study by the same scientist using mice showed pectins inhibiting TLR-2 in doxorubicin induced mucositis (16). Irinotecan has also shown upregulation of TLR-2 associated with IL-1 mediated TLR-2 activation (17). In one of the studies with MyD88 knockout mice, effective downregulation of Irinotecan induced mucositis intensity was seen, indicating that it is the linking molecule for TLR-2 cascade. Many different proteins have been identified in bringing about TLR-2 signalling to cause mucositis, most of which are unclear. The mechanism, though not fully studied, can be traced as follows: LPS like ligands are recognized by the TLR-2, which in turn conscripts the adapter proteins like MyD88 and Toll 1/ IL-1R. This will bring about antiapoptotic effects via P13K/Akt pathways to regulate the junction proteins of the intestinal epithelium. This induces the phosphorylation of p70S6K and downstream ribosomal S6 protein causing inflammation. This will actuate the NFκB production. Further, TLR-2 is found internalized using clathrin coated pits. Interestingly, one of these studies also has reported TLR-2 of having detoxifying capacity via multidrug transporter ATP-binding cassette 1 protein, thus managing the extent of cancer therapy-induced mucosal damage in the GI tract (18). Antibiotic therapy is also found associated with the attenuated TLR-2 activity proving that microbiota and TLR-2 in specific and TLRs in general have a symbiotic regulation. Thus, antibiotic effects can play an important role in regulating TLR-2 and thereby mucositis.

TLR-4

TLR-4 recognizes LPS of the Gram-negative bacteria and is found upregulated in doxorubicin induced chemotherapy where it behaves pro inflammatory (19). It binds to LPS with the help of MD-2 (also called as MDF-2) in the outer layer of the plasma membrane forming homodimer. This MD-2 activated TLR-4 makes up 5 different adapter proteins of the TIR family namely: MyD88, TRIF, TIRAP/Mal, TRIF related adapter molecule, SARM containing protein (20). MyD88 gene has a death domain and a TIR domain. There is a coupling of TLR-4 occurring either to act with MyD88 protein or to bring about its innate function. It requires TIRAP/Mal for both of this. When TLR-4 couples with MyD88, it interacts with IL-1R associated kinase-4 (IL1-RK-4), this activates other kinase members like IL-1RK-2 and IL-1RK-6.

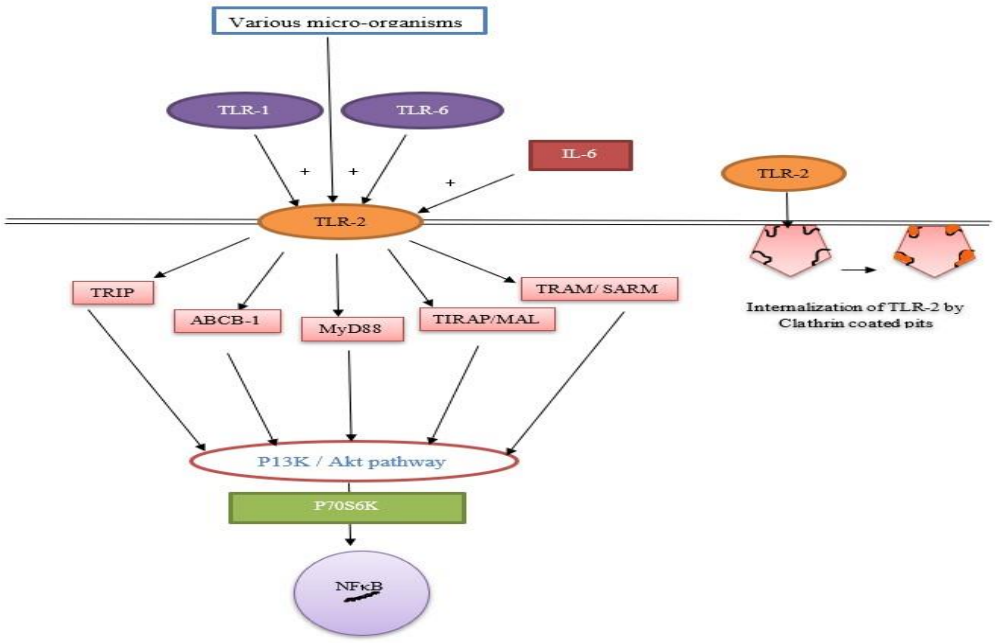

Fig. 2: The intestinal TLR-2 associated pathway: Microbiome derivatives through ligands interact with TLR-2, activated by other TLRs and IL-6, further its effect is mediated by group of proteins

This promotes interaction with TNF receptor associated factor-6 (14). Moreover, studies have also reported that Irinotecan caused upregulation of TLR-4 with block in the IL-6 via regulation of IL-6R expression. Besides, TLR-4 knocked out mice completely masked IL-6 response. This indicates IL-6 plays a prominent role in the cascade. IL-6 is unique in having both pro and anti-inflammatory properties. TLR-4 signals the production of TNFα which in turn along with IL-1β and activation of NFκB, stimulates the production of IL-6. IL-6 can be signalled in 2 forms -cis and trans signalling (21). In cis- signalling, which is an anti-inflammatory pathway, IL-6 binds to IL-6R, makes gp130 protein which will trigger JNK pathway - MAPK activated p38 pathway leading to phosphorylation and activation of STAT3. STAT3 further releases IL-6. In Pro inflammatory pathway which takes place via trans signalling, IL-6 binds to soluble form of the receptor, sIL-6R. This complex also interacts with gp130 and follows the same cascade. But it accumulates T cells and leads to production and migration of TNF-α and IL-1β (22). This supports the observation in study by Lee *et al.*, (19), where inflammatory cytokines and endotoxins leaked into the circulation. It is also reported that Arid5a, a protein domain, plays a vital role in transcriptional regulation of IL-6 mRNA and p38 induced phosphorylation of Arid5a protein in response to LPS. Interestingly, there are also polymorphisms observed in patients where TLR-4 is showing reduced or no activity at all (23). In a recent study, TLR-4 knock-out mice delayed onset of mucositic symptoms and in turn geared up TLR-9 activity in Irinotecan induced mucositis (24).

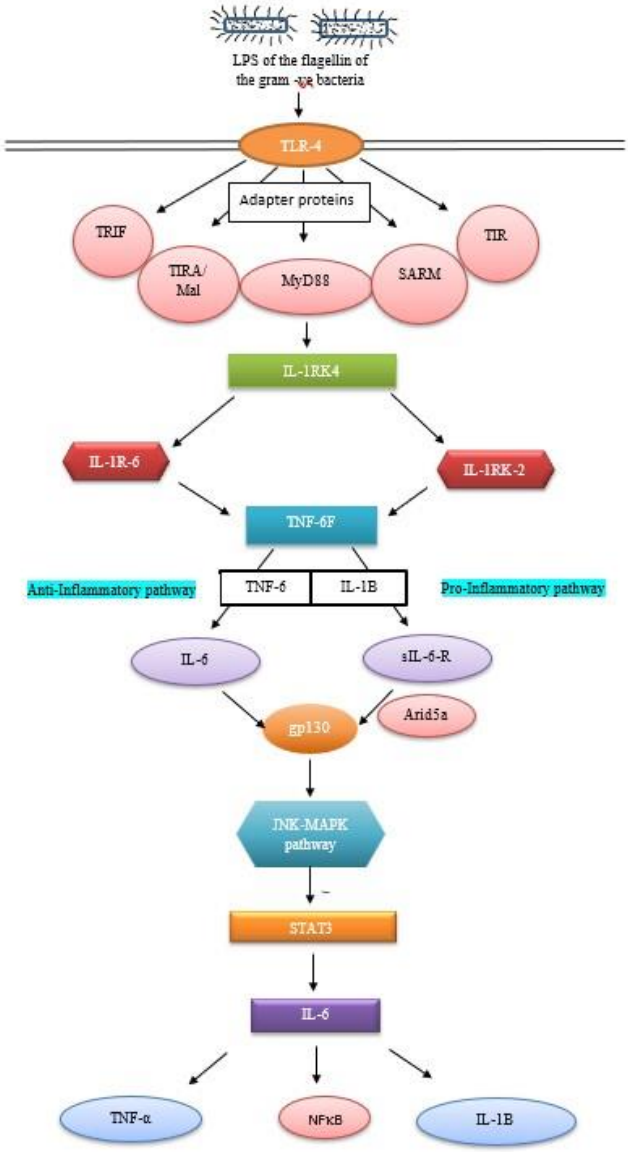

Fig. 3: Intestinal TLR-4 associated pathway: TLR-4 through its adaptor proteins brings about the cascade by the activation of kinase, this further modifies TNF-6 and via JNK-MAPK mediated pathway brings about the changes in TNF-α, NFκB and IL-1β

TLR-5

TLR-5 and flagellin interactions are of great interest in knowing the micro biotic and immunological patterns. It recognizes LPS in general and flagellin in particular (25). TLR-5 is also known to have protective action in various tissues like lungs and liver (26). It is reported that this TLR-5 scrutinizes the localization and makeup of intestinal microbiota to prevent inflammation. It depends on transcription factors MyD88 and TRIF, similar to TLR-4. Moreover, Butyrate-like substances, which are chief fermentation products of the microflora, augment flagellin mediated TLR responses (27). Entolimod, an agonist of TLR-5 showed decrease in TNF-α mediated inflammation, protecting antitumor effects (28). This means TNF-α is expressed during TLR-5 mediated mucosal inflammation. Besides, flagellin activates TLR-5 on dendritic cells leading to the production of IL-22, which acts both as pro and anti-inflammatory cytokine. Expression of TLR-5 on intestinal epithelial cells (IECs) decides the composition and localization of intestinal microbiota (Fig.4). Besides, loss of TLR-5 from dendritic cells down regulated the production of IL-22 and by then resulted in complete loss of flagellin induction. Another study states that TLR-5 agonist mediated by flagellin later activates the transcription factors like NFκB and AP-1. These causes of expression of several antiapoptotic, ROS scavenging genes, can limit the radiation damage in normal cells (29). This can be a new avenue for chemotherapy adjuvants.

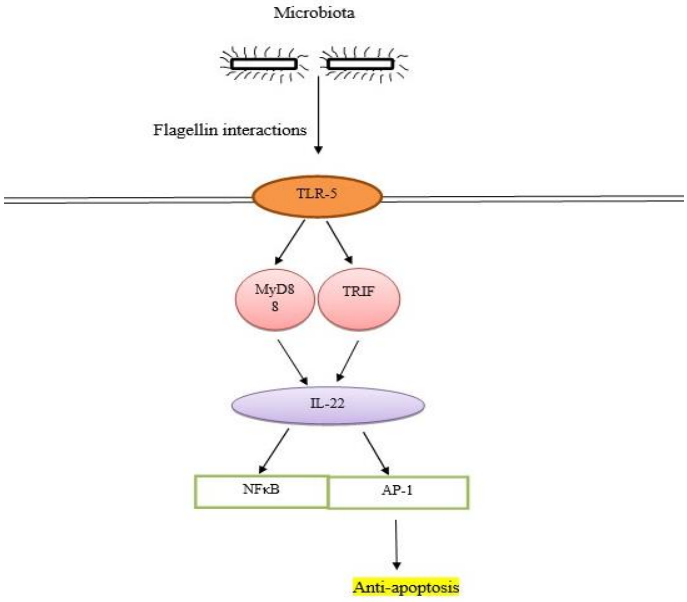

Fig. 4: The involvement of intestinal TLR-5 associated pathway showing anti-apoptotic effect

TLR-9

TLR-9 is one of the PRP which can sense apoptosis. Interestingly, it is seen that deficit of TLR-4 activity shunts the pathway to TLR-9 cascade to ameliorate the intestinal damage(24). The TLR-9 family recognizes cellular components, matrix metalloproteinases, DNA and RNA mediated viral antigens and microbial antigens etc., as ligands and takes DAMPs related pathways. TLR-9 specifically can recognise nucleic acid derived structures of the microbiota. It is activated by unmethylated CpG-DNA of the microbiota and viruses (30). TLR-9 is located in the ER in resting cells and targeted to the plasma membrane through secretory golgi bodies using coat protein complex-II (COPII). At the plasma membrane, AP-2 is recruited for its endocytosis through clathrin coated pits (31). Further inflammatory pathway can be outlined as follows: It can take either of the two pathways: First, it will trigger IRF production through the IRF-signalling endosomes (IRF-SE). Second is activating NFκB through the NFκB signalling endosomes (NFκB-SE). This leads to production of IFN-I. NFκB-SE induces the production of proinflammatory cytokines (32). In the second pathway, AP-3 plays a crucial role in the conduction of TLR-9 cascade. It helps in the formation of lysosome related organelles (LROs) which is further required for carrying out IRF-SE pathway. This AP-3 is regulated by Phosphoinositide -3- phosphate-5-kinase (PIKfyve) that decides the phosphorylation of PhosphatidylInositol (PI) derivatives (Fig. 5). This phosphorylated PIKfyve converts PI-3-Phosphate to PI-3,5-Bisphosphate which interacts with AP-3(33). This helps in TLR-9 and CpG interaction with IFN-SE. PIKfyves are more active in the dendritic cells. It is also being studied that blockage of PIKfyves will attenuate type-1 IFN production suppressing NFkB and IRF-7 pathways (34).

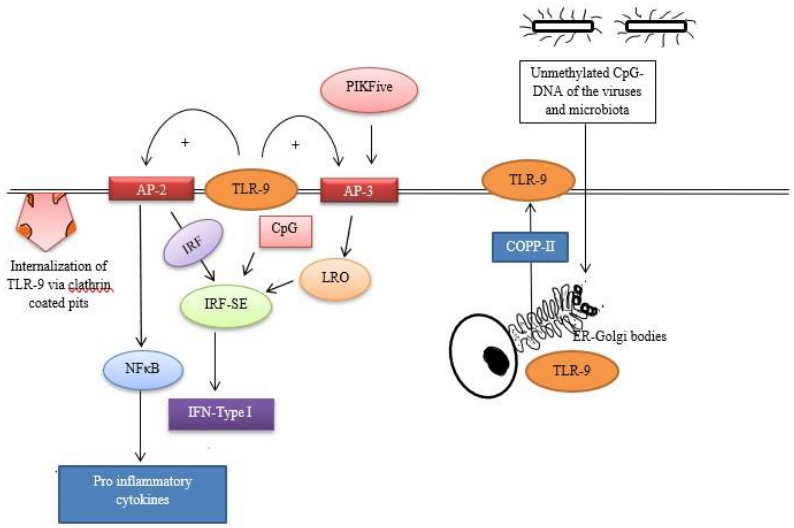

Fig. 5: The TLR-9 associated pathway activates the surface proteins, in turn elicits the IFN, results in increased formation of pro inflammatory cytokines through NFκB

Study by *Hajam et al.*, also supported the involvement of TLR-9 via MyD88 in DSS induced colitis where both nonviable probiotics and active microflora acted in a similar way through their genetic material (35).

CONCLUSION

In summary, TLRs are the tracking molecules which actuate the systemic and inflammatory changes at the molecular level and can serve as ground reporters for the treatment plan and the decision regarding drug load that could modulate the effect of mucositis. Use of TLR agonists and antagonists as adjuvants during chemotherapy may be aimed to attenuate the mucositic vulnerabilities.

CONFLICT OF INTEREST

The authors declare that they do not have any conflicts or competing interests with respect to the review conducted.

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