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Article Reviw :

The Possible etiological role of Bacterial Lipopolysaccharides and Tool Like Receptor 4 in colorectal cancer

Layla Mahmood Saeed// University of Baghdad - college of education for Pure science Ibn AL-Haitham
laila.saeed2202p@ihco. uobaghdad.edu.iq
Dr. Hazima Musa khalel // University of Baghdad - College of Education for Pure Science Ibn Al-Haitham
Hazima .M.K@ihco.uobaghdad.edu.iq
Karkh Education III

Abstract:

One of the diseases that has highly mortality rate among cancer types is Colorectal cancer (CRC), which triggered by variety factors like genetic, environments, nutrition and life style. CRC is increased in Iraq in last years, thus it still under investigations, the present review discussing The Possible etiological role of Lipopolysaccharides (LPs) and Tolle Like Receptor 4 (TLR4) in CRC, The up-regulation of certain TLRs in CRC cells proposed that TLRs has a vital effect in the inflammatory and chronic diseases prognosis that lastly culminate in CRC, the review clarified the contribution of the TLR pathway in the beginning, metastasis and progression of CRC. the physiological role of TLR4 in CRC pathogenesis, and propose novel belong to the effect of TLR4 in immune response (IR) and the microbial community that contributed on the LPs in colitis, human studies have found that response of preoperative systemic inflammatory is prophetic of recurrence tumor after influenc curative resection. Lipopolysaccharides (LPS) are biomarkers of gram-negative bacteria (G^{-V}) membrane surface, which stimulate gut barrier inflammation and dysfunction, it also has significantly implicated in the CRC appearances and development. the CRC progressing and development are stimulated by association of TLR4 and LPs with immune response (IR). These relationships are discussed in present review. We can conclude the vital role of TLR4, LPs and IR stimulated by these factors in CRC.

Key word: LPS,TLR4,Colorectal Cancer .

الدور المسبب للمرض المحتمل لمتعدد السكريد الدهني ومستقبل اشباه العُدد في سرطان القولون والمستقيم.

م.م ليلى محمود سعيد ، أ.د حازمة موسى
كلية التربيه -ابن الهيثم للعلوم الصرفه
كليه التربيه ابن الهيثم / جامعة بغداد للعلوم الصرفة - جامعة بغداد

مستخلص

مرض سرطان القولون والمستقيم أحد الأمراض التي ترتفع فيها معدلات الوفيات بين أنواع السرطان في العراق . يسببه عوامل متنوعة مثل الوراثة والبيئات والتغذية ونمط الحياة. تم زيادته في العراق في السنوات الأخيرة، وبالتالي لا يزال قيد التحقيق، وتناقش هذه المراجعة الدور المسبب للمرض المحتمل لعديدات السكاريد الدهنية (LPS) ومستقبلات شبيه العدد TLR4 لسرطان القولون والمستقيم، واقترح تنظيم بعض TLRs في خلايا سرطان القولون والمستقيم. أن TLRs لها تأثير حيوي في تشخيص الأمراض الالتهابية والمزمنة التي تبلغ ذروتها أخيرًا في سرطان القولون والمستقيم، أوضحت المراجعة مساهمة مسار TLR في البداية، في تطورالورم الخبيث و سرطان القولون والمستقيم. الدور الفسيولوجي لـ TLR4 في التسبب في مرض سرطان القولون والمستقيم، تم اقتراح تأثير TLR4 في الاستجابة المناعية والمجتمع الميكروبي الذي ساهم في متعدد السكريد الدهني و في التهاب القولون، وقد وجدت الدراسات البشرية أن استجابة الالتهابات الجهازية قبل الجراحة تنبئ بتكرار الورم بعد الاستئصال العلاجي. تعد عديدات السكاريد الدهنية (LPS) من العلامات الحيوية لسطح غشاء البكتيريا سالبة الجرام (G، والتي تحفز التهاب حاجز الأمعاء واختلال وظائفه، كما أنها متورطة بشكل كبير في ظهور سرطان القولون والمستقيم وتطوره. يتم تحفيز تقدم وتطور السرطان من خلال ارتباط TLR4 و LPs بالاستجابة المناعية. وتناقش هذه العلاقات في المراجعة الحالية. يمكننا أن نستنتج الدور الحيوي لـ TLR4 و LPs و IR الذي تحفزه هذه العوامل في سرطان القولون والمستقيم .

الكلمات المفتاحية : متعدد السكريد الدهني ، مستقبل شبيه العدد ، سرطان القولون والمستقيم .

1-1-Introduction

Cancer is the main cause of death worldwide (Hasan , 2024). Cancer is a disease in which a group of abnormal cells grow uncontrollably .(Abood, 2018) colorectal cancer CRC is disease included colon and/ or rectum cancer, that reported as one of the highly health disorders in the world, globally it a third most cancer types and second most fatal (WHO, 2021). which constitutes about 10% of cancer mortality. Malignant gastrointestinal tumors such as esophageal, gastric, liver, colorectal, and pancreatic carcinomas are a major cause of cancer-related deaths worldwide.(Rasheed,,2022) One of the Top Ten Cancers is (CRC) (4 th) (Abdullah ,2021).

Its form About 9.4% of deaths related with cancer in 2020 (Ferlay,2021). The evidences expected that the CRC incidence will be more than twice by 2025 in developed nations (Papamichae, 2015). Worldwide, colon cancer ranks third among women and fourth among men, Notably, there were differences in CRC distribution across different countries (WHO,2023).

Obesity and overweight, diet deficient in vegetables and fruits, physical inactivity, and smoking are risk factors for CRC, As a result, the disease was previously mostly seen in long-established developed countries, whose people also tend to exhibit these variables (Renehan, 2008). elevated levels of some chemokines that act as growth factors and can promote cancer development (Neamah,2024). there is significant correlation between the psychological problems related for these patients and one demographic characteristic that was region (residency) of patients (Khalaf ,2013).However, in recently industrialized nations across the world, where the risk was previously low, substantial incidences of CRC have been identified (zhang, 2021). CRC can be categorized as genetic instability, and presence of gastrointestinal tract chronic inflammation like Crohn's disease or sporadic, which about high than 80% of all CRC (Jasim, 2015).several study domestrated the role ofdifferent gene in GC AND CRC. (Rasheed, 2021). Several important molecular alterations that have important function in etiology and progression of colorectal have been widely studied by researcher such as mutations of the KRAS and PIK3 genes in addition to methyla-

tion process like in P16 , MLH1 and BNIP genes also were explored (Musawi,2017)

It has been found that LPS stimulate the release and expression of inflammatory cytokines leading to an acute inflammatory response by activation of Toll-like receptor 4 (TLR4)-dependent pathway[Al-Rikabi,2018]TLR4 identified as a LPS receptor, now it's widely observed that the Coley's toxin pro-inflammatory activity, which consist of different bacterial compositions, like highly immunostimulatory LPS, particular mediated by TLRs linked with immune cells, the TLRs has a significant effect in IRs activation. TLRs identified conserved pathogen-associated molecular patterns (PAMPs) produced in a various microorganisms, as in endogenous DAMPs liberate from stressed or dying cells (Adams., 2009). Based on the stage, upfront surgery is the major therapeutic type, then adjuvant chemotherapy uptake. Several medications have U.S. Food and Drug Administration approval, CRC are linked to right-sided primary cancer, older female and high-grade tumors (Sabry, 2022). radiotherapy, mainly used for the treatment of systemic proliferation of tumor, (Rasoul,2019).

The rate of CRC in male were (8.37%,5.69/100,000 MP), The rate of CRCs in females were (5.89%, 5.31/100,000 FP), in Iraq (2020) the distribution of CRC in Baghdad are 586 cases and the rate is 7.29 %, male 323 and female 263 cases (Iraqi Cancer Registry-2020). The percentage of all CRC cases to total malignancies raised from 3.69% - 6.5% in same period also. While mortality of CRC proportion also raised (1.25 - 1.77) per 10^5 populations (Ibrahem ,2022) . Cancerous cells are stressed cells that express huge amounts of intracellular and extracellular heat shock proteins to correct for distorted proteins. They also directly affect immune cells and stimulate high amounts of cytokines production (Ismaeel *et al*., 2023).

1-2 The biology of toll-like receptors and signal cascade

The toll-like receptors (TLRs) is A category of conserved structure discrimination receptors (PRRs). TLRs classified under the type I transmembrane glycoprotein receptor group With trans-membrane, intracellular C-terminal signaling and N-terminal ligand-recognition domains (Wang et al., 2012) There are 13 TLRs known to exist in humans and mice, several of

TLRs have same form with the animal species, Genes implicated in antimicrobial host defense like those encoding proinflammatory chemokines and cytokines, are induced upon engagement of TLRs with a broad variety of microbial moieties through the intracellular signaling cascades stimulation (David *et al*., 2014). TLR signaling has been the subject of some researches. Firstly, the LBP and CD14 sequential action has triggered the TLR4/MD-2 heterodimer formation in the cell membrane; the intracellular signal can take either ways TLR4/TRIF/IRF3 or TLR4/MyD88/NF-kB . other receptors types including the signaling TIR location-containing adaptor protein (TIRAP), the adaptor proteins myeloid variation initially response gene 88 (MyD88), TIR-location-containing adapter-stimulating interferon-β (TRIF) and TRIF-associated adaptor molecules (TRAM) (Giovannucci, 2006). The intracellular TLR4 unit and all TLR4 adaptor units own Toll/interleukin-1 receptor (TIR) site which involved mutual interplay. MyD88-dependent and TRIF-dependent way are mutually exclusive and competitive (Rychter, 2023). TLR4/MyD88 way starting from the complex $(LPS/MD\text{-}2/TLR4)_2$ found on cell surface, where transduction of TLR4/TRIF begin after internalization of complex in endosomes. It is may be debated the both ways by components that exist selectively on endocytosis. another evidence found that the anti-inflammatory function of several antimicrobial peptides is depended on the TLR4 endocytosis prevention in LPS-activation cells that prevent the TRIF-dependent part of signaling of TLR4 (Shim *et a*l., 2015). Notably, it has also been demonstrate that CD14 have major effect in the complex (TLR4-MD2-LPS) internalization promoting in endosomes (Zanoni *et al*., 2017).

1-3 definition and characterization of Toll-like receptor 4 (TLR4)

Toll-like receptor 4 in human is transmembrane protein transcript form TLR4 gene located at 9q33.1, about 95 kDa from this gene is expressed, it compose of 3 exons, it is one type of the toll-like receptor group, that regarding to the family of recognition receptor (PRR) pattern. The IRs are initiated via members of the Toll-like receptor (TLR) (Abood, 2023).

Mainly, TLR4 is produced in immune cells of myeloid origin, like dendritic cells macrophages and monocytes (Vaure, 2014). also produced in

low concentration in other cells like placental cells, endothelium, epithelium and beta cells. some myeloid cells produced plasma membrane-anchored CD14 in a high level, that assist TLR4 stimulation by LPS and regulate the subsequent LPS-stimulate internalization, TLR4 essential for receptor degradation and signaling (Sabroe, 2002; Yang , 2015).

TLR4 signaling pathway has carcinogenic consequences in some studies, A typical characteristic of CRCs is highly production of TLR4 and IL6, which is linked to a poor prognosis (Nagao, 2016). Yesudhas et al. (2014) investigated the production of TLR4and MYD88 in CRC to illustrate the significance TLR4 signaling in colon carcinogenesis, They proposed the elevation production of TLR4 and MYD88 is related to metastasis in liver and is separated indicator of a weakness prognosis in CRC cases to clarify the significance of TLR4 signaling in colon carcinogenesis.

1-4- .Intestinal Homeostasis and TLRs

it is suggested that interactions between intestinal microbes, environmental factors and host genetic predisposition are required to trigger irregular immune responses that are participated in provoking chronic relapsing and remitting inflammation [Abdul-Hussein,2021].The toll-like receptor activation is a process that combats microbial infections while sparing the host cell. Typically, inflammatory mediators, antimicrobial peptides, the antigen presenting cells maturation, in addition to the activation of pathways for tissue repair and cell survival are used to achieve this (Metzatof, 2008). TLRs are mostly found on the endosomal vesicles and in the IECs basolateral surface where they are only weakly expression of TLR inhibitors such as A20, interleukin 1 receptor association kinases 3 (IRAK3) and single IL-1-related receptor (SIGIRR); these inhibitory molecules block TLRs to IR via continuous interaction and fostering the anti-inflammatory phenotype of leukocytes (Abreu, 2010). Defects in intestinal homeostasis are seen in SIGIRR-deficient animals, and these abnormalities are linked to the overexpression of inflammatory mediators and the microbiota. Remarkably, these flaws also make the mice treated with azoxymethane and dextran sodium sulfate susceptible to colitis and colitis-connected colorectal cancer. While

increased TLR activity interferes with TLR2 and TLR4's ability to recognize intestinal microorganisms, TLR signaling is essential for IEC homeostasis and repair controlling (Xiao, 2007). PTEN gene mutations caused the etiology of CRC as there were genetic alterations in bowel inflammation and CRC patients [Mahood,2016].

1-5- Toll like receptor 4 role in colorectal cancer development

CRC complexity is primarily involved to environmental factors, where a minor role represented by genetic factors. The CRC risk factors are food-with pollution, mutagens, chronic intestinal inflammation and certain commensal bacteria, (Terzic, 2010). Globally, CRC observed in the right ascending colon has symptoms being blood in the rectal bleeding or stool. In genetic factor, inherited colon polyps involved to CRC progression (Adelstein, 2011). CRC can injury the host immune system through the period of their proliferation, to stimulate against CRC promises to development treatment research (Evans ,2003) .

The extracellular location of TLR4 is consist of 22 leucine-rich repeats (LRRs), that mediate receptor dimerization and LPS discrimination, a transmembrane domain; and the Toll/IL-1 receptor domain (TIR) that is important for transduction downstream signal of TLR4 alone didn't associate LPS directly and needs the co-receptor MD-2. MD-2 is binding to the TLR4 extracellular domain and binds LPS directly, giving LPS responsiveness on TLR4 (Ohto, 2012). the innate immune system have a strong process in the conserved TLRs form that can estimate the signature approach of invading organism to protect the host cells (Kawai ,2010).

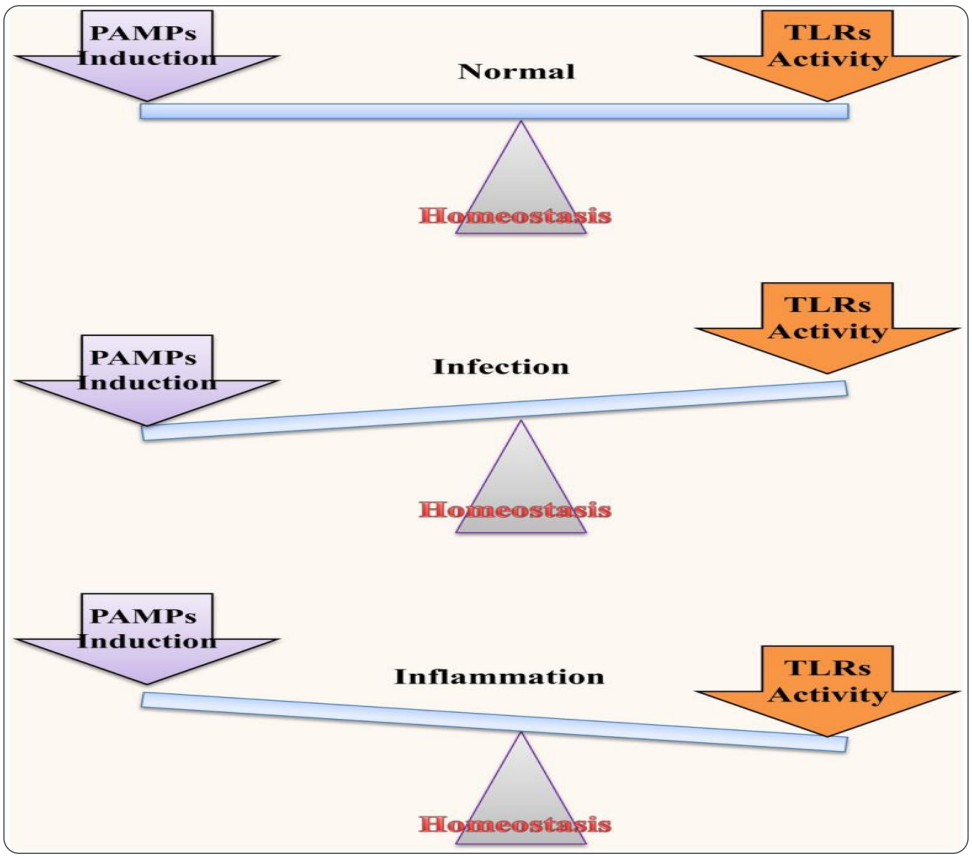
TLRs not recognize invading microbes only but detect intracellular anomalies and IRs quantities, thus have vital impact in the human immune system homeostasis (Rakoff-Nahoum, 2008). The TLRs abnormal activation can imperil normal physiological pathways and lead to some inflammatory diseases as well as, autoimmune diseases and cancers (Keogh,2011).

TLRS are expressed as ubiquitously, in spite of the variation expression level bases on the conditions and the tissues. Also, stimulate the TLRs expression has seen when ligands connect to their cognate TLRs (Shi,2011). Report in the last years has demonstrated different functions, intermedi-

ate units, and ligands correlated with TLRs. a good documented association between the cancer development and progression with TLR-induced inflammation .On the other hand, TLRs are also known to have essential effect in CRC that impact in the rectum and large intestine. This location is highly common by intestinal microbes, focusing the essential effect of TLRs in the CRC pathogenesis (So *et al*., 2010)**.**

1-6 Contribution of TLR4 in Colorectal development

In spite of IECs are in strong relation to LPS, they aren't made an IR on the normal flora in normal conditions. Whatever, the coexistence disturbance in the diseased state between IECs and bacteria lead to an inflammatory response, it leads to question: how much and when inflammation should be elevated to equilibrate the bacterial infection (Fig .1). some reports have been carried out to treat this dilemma (Leaphart, 2007). Such as, IFN-γ and IFN-α are called to LPS response increasing in IECs, that is associated to the TLR4 and MD2 production (Abreu, 2001). In addition, the chronic LPS activation culminates in TLR4 level reduction and elevated inhibitory proteins production. Moreover, a conflicting study elucidated that long time LPS exposure is not change TLR4 production. hypoxia and some endotoxins are found to be frequent in the inflamed intestinal lining, that lead to TLR4 production induction (Leaphart, 2007). Other study found TLR4 level elevation from the CRC patients mucosa of different sexes and ages like from a different of CRC (Doan,2009). Moreover, a study found that TLR4 level is needed for polyp formation and dysplasia. This observation is proportionate with finding implemented in knockout TLR4 gene in mice (Fukata, 2006). Globally, these finding exist a clear linked between CRC development and TLR4 .



(Fig.1) Homeostatic interaction between TLRs and microbiota. (Yesudhas, 2014).

The TLRs localization is heterogeneous and are varied from the cell membrane in the human (Blasius, 2010), based on the of pathogen-associated molecular patterns (PAMPs) localization. Its consisted of the three regions: ectodomain [leucine rich repeats (LRR)] which estimated PAMPs, a trans-membrane location, and a cytosolic toll/interleukin-1 (IL-1) receptor (TIR) region linked with adaptor unit (like MyD88/MAL and TRIF/TRAM) to increased downstream signaling, the Ligand linking stimulate the TLRs dimerization, assistance the binding of adaptor unit.

1-7- TLR4 Crosstalk in Progression of colorectal cancer

TLR4 is excessive expression in the CRC with liver cancer (Laird, 2009). In response to LPS association, the TLR4/MD2 structure high activation stimulates the phosphorylation of protein kinase B, that activates the β1 integrin function. This structure interaction between pathways stimulates the adhesiveness and CRC metastatic attitude (Sheng, 2003). The AKT phosphoryla-

tion enhancement can be stopped by eritoran, PI 103 [a phosphatidylinositide 3-kinases (PI3K) inhibitor], or anti-β1 integrin antibodies which found to improvement and metastatic attitude of CRC (Somanath, 2007), known that the PI3K/AKT signaling process is stimulated by TLR4 in response to LPS association and have vital effect in the CRC progression. Moreover, LPS has been found to stimulate the urokinase plasminogen activator (uPA) system expression via TLR4 and NF-κB in CRC. Through progression of tumor, vital extracellular matrix interplay observe, in which uPA and the its receptor level and function assist the CRC growth and metastasis (Killeen, 2009). contrariwise, TLR4 inhibition, NF-κB, or the uPA system can weaken progression of CRC.

1-8- TLR In colorectal cancer Prognosis

the high level of the TLR4/ MYD88 signal was related to weak prognosis of CRC (Brunner, 2010). In the tumor condition, high TLR4 level make it as a possible test of CRC progression . TLR4 level in stromal fibroblasts is linked to weak CRC prognosis, thus, TLR4 level in fibroblasts might be benefit for CRC prognostic biomarker (Eiró , 2013). A study found that the STAT3 and NF-κB deregulated activation is a prevalent characterization of gastrointestinal tumor and invariably associated with weakness prognosis; NF-κB and STAT3 are downstream signal transforming key of TLR groups and IL-6 .

TLR-specific stimulation of NF-κB in CRC and especially in tumor-starting cells may support more cancer development by perpetuation of signaling from tissue repair processes and inflammatory, with resulting self-renewal of pluripotent cancer cells .

In CRC, high TLR4 level is found in all cancer compositions like the endothelial, epithelial and stromal layers (Cammarota, 2010). Otherwise, the expression level varies according to the cancer type. In spite of all TLRs produced at the low basal mRNA level, IECs can up-regulate the TLR level, according to the inflammatory signals or other induction (Fukata, 2007). Another report elucidated a TLR4/MD2 low level in the normal lamina propria and epithelial cells in human and that is strong link with the TLR4/MD2 level estimated in different epithelial cell lines (Abreu, 2003). These reports documented the opinion of any chang-

es in the inflammatory circumstances prevalence or the luminal bacteria community may stimulate the robust and TLR signaling, to facilitate the inflammatory responses starting in IECs (Katakura, 2005) .

1-9- TLR agonists in cancer immunotherapy

LPS ability to stimulate and promote proliferation of T cell which has a huge role to trigger APCs and generation of large quantity of proinflammatory cytokines induction that enhance nonspecific T cell activation and expansion (Yadav, 2006). Moreover, the T cells CD8 and CD4 can also stimulate TLR4 activation with LPS. The first work of TLR4-activating T cells was found murine IL-2-dependent cytotoxic T cell line, the CT6 was proliferated in LPS response, TLR4 linking on human CD8 T cells has found to the induction of IFN (Vogel et al., 1983).

The murine CD8 T cells didn't observe TLR4 express or its supplement protein CD14 and it doesn't TLR4 triggering responding. In contrast with naïve murine T cells, that do not stimulate to some TLR agonist, in vitro the LPS addition to naïve CD4 T cell intensify their survival and proliferation (Reynolds, 2012). An analysis via subset of CD4 T cell which produce and reply to TLR4 signaling demonstrate that TLR4 mRNA was produced at once by murine Th17 CD4 T cell sets in compare with Th1 and Th2 (Reynolds, 2010). moreover, in murine CD4 T cells, LPS decrease INF concentrations unless elevated IL-17A expression (Gonzalez, 2010). These events on CD4 Th cells were produced by reduction MAPK stimulation. TLR4 activation on CD4 T cells has elucidated to increase intestinal inflammation and have significant effects in stimulation colitis, focusing the strong impacts that TLR activation on T cells can stimulate. An effect for TLR4 signaling in CD4CD25 TRegs isn't well understood. clarified that LPS can stimulate CD4CD25 TRegs, their reproduction activation, and assist its immunosuppressive function. This is contrast to the preventing impacts that TLR1-TLR2 activation has on TRegs. Furthermore, Zhu et al. (2011) observed that TLR4 connection on TRegs with HMGB1 decreased the levels of CTLA4 and forkhead box p3 and IL-10 expression limitation. furthermore, stimulating TLR4 on TRegs stimulate signals by TRIF, where TLR4 activation on non-TReg T cells occur to observed by p38

MAPK and MyD88 signaling primarily (Caramalho, 2003). These disparate findings may have found from the vary TLR4 agonists using or from disassociation appear from genetic variation.

study has been suggested that TLR4-driven inflammation might have significant impacts in tumor: where chronic inflammation can assist tumor growth and development (Wu, 2018) stimulate trigger of the TLR4 axis support antitumor reply and is related to a positive tumor growth prognosis, cancer metastasis reduction and suppression (Yahiro , 2020). According to these results, TLR4 signaling pathway induction and/or amplification were proposed as an influence medication strategy in specific tumor (Shetab,2018).

Lastly, MPL® has been documented for some human viral vaccines uses (Cimica , 2017). Likewise, substantial batch-to-batch differentiation in the vaccine-grade MPL® adjuvant and contaminate CU3ions with underacylated TLR4-antagonizing lipid A variants cases numerous restriction (Wang, 2020). It is found that TLR4 moderate stimulation by exogenous PAMPs comprises essential pathways of defense versus viral hitting in an immunocompetent host by guarantee the specific pro-inflammatory signaling pathways induction (Shinya, 2011). thus adjuvants targeting TLR4 can support other pros by showing an intrinsic protective response goal at the immunological memory stimulation. Thereby, there is a large investigation for the development of more impacts TLR4 agonists other than LPS as Very lastly, TLR4 has proposed as an efficient therapeutic goal for drug uptake (Bachtell, 2015) and major depression disorders (Liu, 2014; Wu, 2015), like amyotrophic lateral sclerosis (De Paola, 2016). Possible using of TLR4 antagonists in medication of peripheral neuropathic pain has demonstrated (Thakur, 2017).

in a study and pose a hurdle, The concept that systemic TLR agonist-based therapies are ineffectual in developing optimal cancer medications are mention by researchers (Guha, 2012). Studies were found that tumor-specific human T cells cloned to express the TLR5 ligand at the cancer location stimulate antitumor responses and prolong survival of T cell. also the Autocrine TLR5 signaling in T cells lead to the different cytokines/chemokines production at the location of tumor that more proved antitumor capacity by

provide T cell and DC infiltration (Li, 2007).

1-10- Therapeutic Potential of TLR4 Antagonists

A study has been found that Eritoran protects mice administrated lethal influenza by OxPAPC-dependent was stimulation blocking of TLR4 and damage TLR4-mediated signaling which may be prevented by TLR4 antagonists interplay with MD-2 (Shirey, 2013).

Another chromatin-associated protein-derived DAMP, HMGB1 that produced by dying cells through viral hitting may be sensed via the TLR4 co-receptor MD-2 (Shirey, 2021). The HMGB1 activated pro-inflammatory responses as well as of LPS and thus proved the important role of the medication influence of TLR4/MD-2 antagonists for viral infection features. It is suggested that the TLR4 axis is contributed in the estimation impacts of West Nile virus, respiratory syncytial virus, filovirus and influenza virus (; Sabouri, 2014; Younan, 2017; Olejnik, 2018).

Morbidity and mortality companied with acute viral infection is correlated with huge inflammation stimulated by chemokines and pro-inflammatory cytokines high level that can cause life-threatening problems likes circulatory shock, acute respiratory distress syndrome, and myocarditis. additionally, the TLR4-mediated pro-inflammatory signaling up-regulation have significant impact in the SARS-CoV-2 pathogenesis (Choudhury, 2020; Guan , 2020; Aboudounya, 2021).

Some reports documented the TLR4 role in the skin defense mechanisms regulation during cutaneous tumorigenic inflammation (Dickinson, 2018). the responses of TLR4-driven Pharmacologic inhibition stimulate the skin's photoprotective capability, attenuating cutaneous stress signaling (Blohm-Mangone, 2018).

2- Lipopolysaccharide role in colorectal cancer

2-1 Chemical Structure of Lipopolysaccharide (LPS)

Lipopolysaccharide or LPS are essential outer surface composition of G-V bacteria. Its huge amphipathic glycoconjugates which mainly compose of a hydrophobic or lipid region linked to a distal polysaccharide and a core oligosaccharide (Farhana, 2023). These units are called as lipogylcans based on the sugar molecules and lipid (Cao, 2023) . The LPS are consist

of Lipid A the hydrophilic core polysaccharide, hydrophobic domain and O-antigen.

The lipid A compositions are different according to organism and are vital in specific pathogenic bacterial features (Allen, 2019). Inherent to G-V, LPS gives bacterial cell integrity and a process of bacterial interplay with other surfaces (Li , 2017). Some LPS molecules are produce a strong pro-inflammatory of immune system in mammals, it's also thermo stable. Since LPS different types are found in variant genera of G-V, LPS is benefit for serotyping G-V (Maldonado, 2016), particularly, O-antigen conveys serological discrimination to the bacterial types. M oreover, the size and components of LPS are largely dynamic among bacterial types. According to unique features, LPS has considerable report to complex composition understanding, transport, biogenesis and assembly

2-2- Role of Lipopolysaccharide in TLR4 signal activation

LPS is the major component of the outer layer of the cell membrane in gram-negative bacteria (Salih, 2019) The human immune system discriminate Lipid A molecules in LPS, that is produced by dividing cells in soluble matter, in lysed cells after autolysis of Bactria, or by complement stimulation killing or phagocytosis and antibiotics effected. The early response is identification and association the lipid A composition by the host cell TLR4. The highly immunogenic Lipid A is generated by *Salmonella* and *E. coli* , while *Yersinia spp.*, changed the acylation extent of lipid A after hitting to generate LPS of low immunogenicity. Notably, LPS making poor immunogenicity is bacteria to IR of host avoidness and intracellular survival increasing (Bertani, 2018). Features of G^{-V} bacteria are Represented as an envelope has two membranes: an inner (IM), and an outer membrane (OM) .Therefore, in G^{-V}, the OM used as first defense line against threats, the OM of most G^{-V} is not a phospholipid bilayer, it is strongly asymmetric bilayer, in the inner part consist of phospholipids and LPS unit in the outer part (fig.2) (Silhavy, 2010).

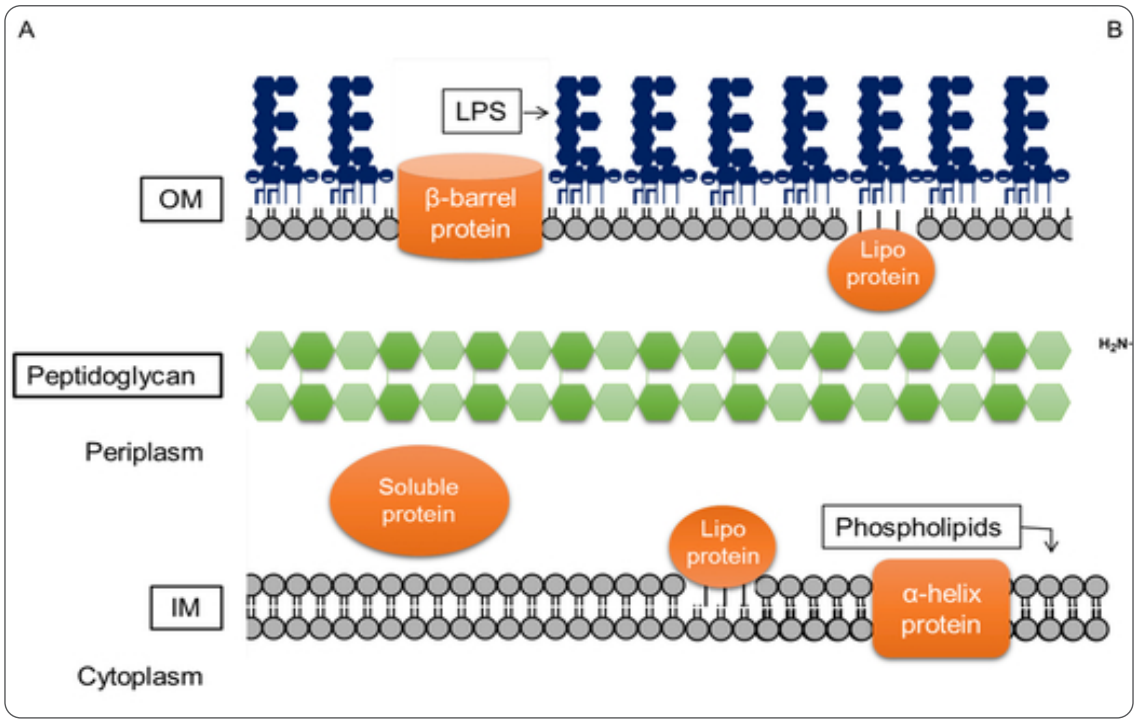


Fig. 2: (structure of the Gram-negative cell envelope (Silhavy, 2010)

LPS are the molecular components known as endotoxins. LPS are existed in the external membrane of G^{-V}. Lipopolysaccharide (LPS) stimulate inflammasome, a hydrophobic lipid domain and hydrophilic core polysaccharide chain; and a side chain of repeating hydrophilic O-antigenic oligosaccharide. LPS are also protected Bactria against lipophilic and antibiotics bile salts (Yoshioka, 2001). LPS was suggested to have antitumor activity in some studies . Several reports demonstrated the LPS effect in gene expression in CRC, LPS stimulate TGFβ and HGF generation stimulate NFkB (NFkappaB) stimulation and the migratory capability increasing in CRC (Liu ,2017).

A study implemented by Yuan *etal*., (2013) who demonstrated a55molecular A High LPS level increased TTP family gene expression in CRC, LPS also elevated Glucose transporters (GLUT1, GLUT2 and GLUT3) mRNA concentration in the CRC, assumed that LPS able to elevate glucose transport into CRC (Cao,2008;cao,2013).

The LPS trigger macrophage damages and inflammation by transcription factor NF-κB. P65 and NF-κB subunit precipitate in the nucleus and can induct nuclear Snail by associated to

genes cluster promoters (Bhatt, 2017). P65 stabilizes Snail and stimulate migration and invasion after TNF-α treatment in breast cancer (Wu, 2009). The glucose flux controlled by Snail by preventing fructose-1,6-biphosphatase and phosphofructokinase, platelet (Kim, 2017). NF-κB effect in glucose metabolism, such as in macrophages, transcription regulation of hexokinase 2 (Gao, 2017).

Some reports have elucidated that inflammasomes and glycolytic enzymes may react to each other. The Hexokinase 1 in the glycolytic pathway is catalyzing the first step activation of NLRP3 inflammasome. NLRP3 inflammasome activate and subsequent IL-1β level modulate glycolysis by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (Finucane ,2019). The Warburg effects (in aerobic circumstances cancer cells prefer metabolism by glycolysis) has been found in tumors. the consumption of glucose are elevated and up-regulation of lactate generation which are glycolysis characterization.

2-3- LPS-TLR4 binding in CRC enhancement

Some studies found in some types of cancer including CRC, LPS may stimulate or involved to tumor progression, carcinogenesis, invasion and metastasis (Liu,2010), according to gut flora and gut barrier changings, LPS destroy was elevated in the CRC cases intestine and portal venous blood (Liu et al., 2017). that proposed impact of LPS in CRC carcinogenesis. Typically in CRC metastasis, LPS can stimulate different ways, like cell adhesion to the ECM, ECM degradation belong to detachment and invasion (Panyathep, 2020). Likewise, in the tumor microenvironment (TME) LPS might stimulate EMT(li *etal,* 2014).the phenotype of mesenchymal cells and fibroblasts of epithelial cells were acquired and their concentration of cell-to-cell adhesion was reduced, which stimulate CRC progression .The In -vitro study have suggested that LPS induced adhesion molecules expression in colon cancer cells (Simiantonaki ,2002). Moreover, LPS elevated liver metastasis of CRC cells in vivo (Hsu, *etal,* 2011).

LPS stimulate the CRC cell invasion and adhesion, in the absent of TLR-4 preventing antibody. (Ikebe, 2009). LPS support the tumor cells avoid immune surveillance by the IL-6, IL-12 production, stimulate nitric oxide synthase like via the antiapoptotic pro-

teins level, such as X-linked inhibitor of apoptosis (Cammarota *etal*, 2010). Reports have also proposed that the TLR-4 chronic activation from microorganism invasion stimulate oncogenic influence in the host, during the NF-κB and cyclooxygenase-2 chronic activation (Thiery, 2006).

The LPS of Bactria is deleterious agent that can involve to the CRC development when dysfunction of gut barrier was happened. LPS-activated TLR4-related inflammatory signaling ways have significant role in cancer metastasis and invasion, affecting features like the low CRC survival rate (Wei *et al*, 2020). in some cancer types LPS is the primary TLR4activator, like liver, pancreatic and colorectal cancer. Based on the 116 CRC patient's analyses, TLR-4 high expression was related to metastasis a high rate and thus belongs to weakness prognosis. (Cammarota *etal*, 2010). Data Analysis have proven role of LPS in CRC cell adhesion and metastasis mediated TLR4 inflammatory signaling pathway (Thiery, 2006).

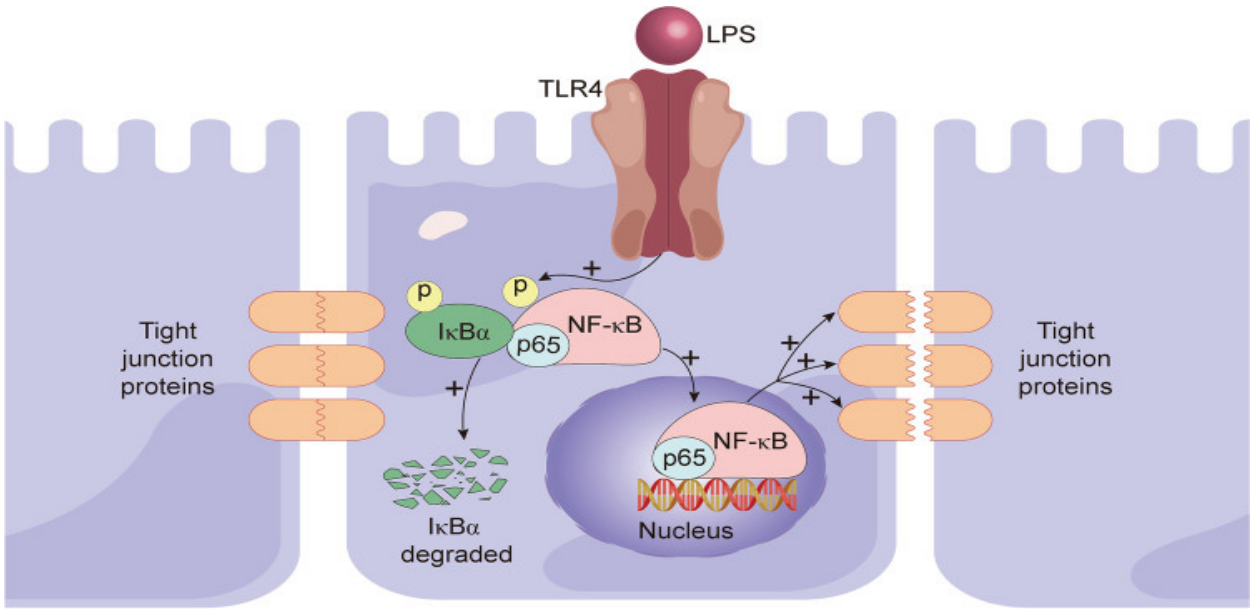
LPS-induced inflammation raises metastasis possibility in tumor. NF-κB has an important role in the inflammatory response (DiDonato *etal*., 2012). LPS-stimulate intestinal epithelial cell inflammation raised adhesion molecules expression by the NF-κB pathway. LPS elevate metastasis capability by different NF-κB-related ways. like, the LPS-elevated HK3 level by signaling pathway of NF-κB/Snail/HK3 activate CRC metastasis (Wux *etal*., 2021) LPS activated the CRC invasion and metastasis by the NF-kB signaling pathway activating via the SDF-1a/CXCR4 axis (Liu, 2017) therefore, CRC cases with high level of CXCR4 chemokine receptor were correlated with liver metastasis and receptor expression poor prognosis (Gao, 2017).

In spite of IECs which in closed relation to LPS, they do not form an IR against the normal flora in normal conditions. Likewise, in the disease, the coexistence disturbance between bacteria and IECs result to an inflammatory response, these lead to study the inflammation amount to be elevated to equilibrate the bacterial infection, several reports have been demonstrated to prove dilemma (Simiantonaki, 2002). IEC can influence effect innate and adaptive immune cell function (Hasan.2021). .like, IFN-α and IFN-γ to raise the LPS response in IECs, that is directly associated to TLR4 and MD2

production (Hsu, 2011). Additionally, continuous LPS activation culminates in TLR4 level reduction and elevated level of inhibitory proteins (Li, 2014). On the other hand, a study elucidated that TLR4 production doesn't change by long-term LPS exposure. furthermore, hypoxia and several endotoxins are found to be common in the inflamed intestinal lining, and stimulated TLR4 production. Hung et al. (2015) found high TLR4 expression from CRC mucosa in different ages categories and cases sex as like from CRC cell lines.

2-4 The Function of LPS in Inducing Dysfunction of the Gut Barrier

Tight association TJs and adherens junctions (AJs) play significant impact in the intestinal barrier integrity maintaining Intestinal barrier damage and increased intestinal permeability result from modifications to AJs and TJs or from the incompleteness of the mucosal layer (Wei,2020). As demonstrated by experiments, LPS has the ability to cause inflammation and decrease epithelium in intestine occludin and claudin-1, that is crucial elements of Tight junctions (TJs), that break down the gut barrier (He et al, 2019) In terms of mechanism, LPS influenced the malfunctioning of the gut barrier through the TLR4/NF-κB pathway of inflammatory signaling. This pathway contributed in the phosphorylation of IκBa and p65 in the cytoplasm, which was facilitated by the activation of TRL4 by LPS. P65 moves into the nucleus, causing TJ disruption as IκBa breaks down.]]Thirteen Research has demonstrated that G^- bacteria like ***Serratia***. marcescens and Escherichia coli may regulate the intestinal barrier, To sum up, these illustrations demonstrate that intestinal LPS has the capacity to compromise the gut barrier (Fig. 3)



(Fig- 3) TLR4/NF-κB pathway-mediated tight junction destruction is how LPS induces disruption of the gut barrier. (Li,2023).

The dysfunction of gut barrier and LPS found to impact in CRC progression during an inflammatory IR increasing. the gut barrier Changes can stimulate IRs and tumor-related inflammation while intestinal inflammation stimulate gut barrier disturbance and the CRC increasing susceptibility. Other report is needed to clarify the processes by which bacterial LPS and gut barrier dysfunction contribute to the CRC progression.

2-5- LPS role in Carcinogenesis and Metastasis regulation in CRC

Some evidences find that LPS implicated to carcinogenesis, progression of tumor, invasion, and metastasis in some cancer type, like CRC. CRC patients base on the gut microorganism and gut barrier changes, the concentration of LPS damage were elevated in the intestine and portal venous blood versus control group (Kim, 2017) which proposed that LPS has an impact on CRC carcinogenesis. Particular in CRC metastasis, furthermore, LPS can stimulate via several approaches, as well as cell adhesion to the ECM, detachment according to ECM disruption, and invasion. (Tomasello, 2014) furthermore, LPS in TME can stimulate EMT. Through EMT, epithelial cells needed

the mesenchymal cells phenotype and fibroblasts and decrease their concentration of cell adhesion, more stimulation CRC progression. (Wang, 2003) In vitro tests have documented that LPS induced CRC to express adhesion molecules LPS causes liver metastasis increasing of CRC in vivo (Li,2023).

The LPS lipid A moiety, which fixed LPS to the outer membrane of G^{-v}, is involved of the LPS immunostimulatory function (Lei, 2021). The acyl chains of lipid A, detection the agonistic function of lipid A. like *E coli* lipid A is hexa-acylated and represented a strong agonist for all mammalian cells, while the precursor of *E. coli* LPS (tetra-acylated lipid Iva) represented as an agonist only for several mammalian species (Garcia , 2019; Wong, 2018). Moreover, deletion of lipid A phosphate group causes endotoxic activity reduction (Nicholson, 2022; Low, 2020).

The activation of mammalian cells LPS happened during a sequences of interplay with some proteins like the LPS binding protein (LBP), MD-2, CD14 and TLR4 (Feams, 2006). LBP is a soluble shuttle protein that linked directly with LPS and assists the binding with CD14 (Kobayashi, 2002). CD14 is a protein, glycosylphosphatidylinositol-anchored, that presences in a soluble form. CD14 assist the LPS transfer to the TLR4/MD-2 receptor structure and changes LPS detection (Hardy, 2004). MD-2 is protein found in a soluble state that non-covalently related to TLR4 but can make a complex with LPS in the absence TLR4 (Cherry , 2006). In spite of suggestion that TLR4 can directly link to LPS, TLR4 can stimulate the association of LPS to MD-2 (Kurt-Jones, 2000).

After LPS detection, TLR4 undergoes oligomerization and bring its downstream adaptors by interplaying with the TIR domains, That consist of three conserved domain, that mediate interplay between the signal transduction adaptor proteins and TLRs. The TIR domain of TLR4 is critical for signal transferring, regarding to TIR domain point mutation which abolish the response to LPS (Bowie , 2005). reports using knockout mice has demonstrated important roles of adaptors in TLR4 signaling. MyD88 was first found as a myeloid differentiation primary response gene .Then it proposed to be the important adaptor in the interleukin-1 receptor signaling pathway .Regarding to the IL-1R and the TLR

has TIR domains, researches were implemented to estimate whether MyD88 was contributed in TLR-mediated signaling pathways. While mice with MyD88-deficient were observed to be resistant to LPS-stimulate septic shock, and macrophages with MyD88-depletion failed to express proinflammatory cytokines after LPS activation, in spite of the possibility to activate NF-κB (Beutler,2003). A study has supported signaling pathway of tremendous insights into LPS/TLR4. The facts from this pathway also indicated models for which other TLR signaling pathways can be controlled. besides improper controlling of LPS/TLR4 signaling has a vital to massive inflammation induction and lead to disorders in chronic inflammatory or acute sepsis (lu, 2008).

Conclusion

The CRC development is highly complex, several reports found that the gut microbiota involved to CRC. The studies demonstrated that the microbiota composition elucidate the vivid variance in microbial community. thus, the changes in LPs and microbial population have an essential role in CRC development and progressing.

TLR4 is a crucial receptor that discriminate LPS and biological behavior induction to defense against bacteria, more investigation on the of immune cell characteristics affected by LPS, like their role in CRC cell lines. The linked between development of cancer and LPS exposure is still under investigation. It seems likely that more details about TLR4 signaling, G^{-V} bacterial infections will be given more answers about cancer growth and metastasis questions. It is also produced in different cancer cells and its function, proliferation and invasion, which suggested high of production in tumor tissues predicted poor prognosis. As the TLR4 activator, Colon bacteria implicated to a large LPS amount which can stimulate CRC metastasis. TLR4 detection pathogen ligands, like LPS endotoxin from *E coli* and mediates signaling to IRs initiation. It stimulate signals contributed for genes activation that important for efficient host defense, particularly proinflammatory cytokines and discrimination represents mediator a link between the acquired and innate-immune systems. LPS-stimulate TLR4 signaling in CTC implicated to their capability of adhesiveness and metastatic and prevent signaling pathway may stimulate to be efficient to eradi-

cate distal organ metastases. Further work may consist of clinical and preclinical trials of the therapeutic agents used.

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