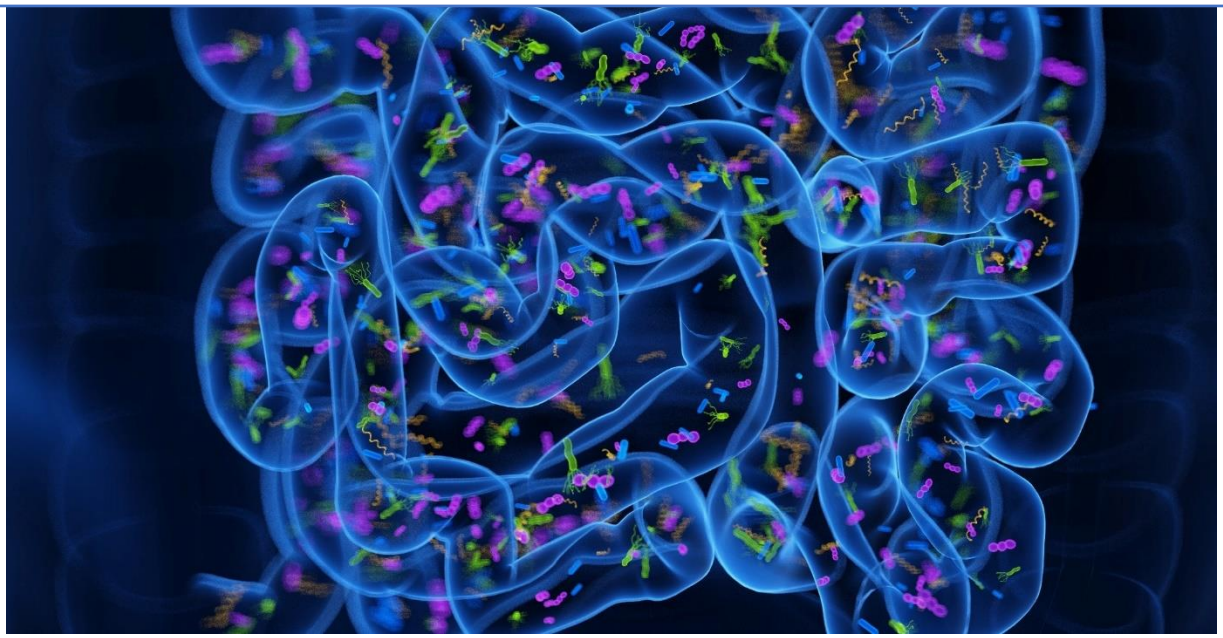

The role of microbiota-derived peptidoglycan fragments in diseases and therapies

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Abstract

The human gut microbiota, inhabited by diverse microorganisms, including bacteria, plays a pivotal role in maintaining our overall health. In its normal state, the human gut microbiota exhibits stability, resilience, and a mutually beneficial relationship with the host. Furthermore, it plays a crucial role in various physiological processes, such as metabolism, immune function, and nutrient absorption. Peptidoglycan fragments derived from the human gut microbiota, acting on immune receptors, are important in preserving this symbiotic relationship, but disruptions in this balance can lead to the onset of various diseases. This literature review aims to explore the potential link between peptidoglycan fragments and the development of different diseases. Additionally, it delves into the therapeutic applications of peptidoglycan. It has become evident that disturbances in peptidoglycan sensing, particularly involving NOD-like receptors (NLRs: NOD1 and NOD2) and peptidoglycan recognition proteins (PGLYRPs), contribute to intestinal dysbiosis and the emergence of several diseases, including Crohn's disease, neurodevelopmental disorders, and cardiovascular conditions. Notably, these disruptions also impact the development of cancer, especially colorectal cancer (CRC). However, peptidoglycan fragments exhibit a dual role, promoting tumorigenesis while also inhibiting it, for instance, by enhancing the effectiveness of PD-L1 anti-tumor immunotherapy. Consequently, peptidoglycan fragments used as therapeutics to enhance the efficacy of cancer immunotherapy hold promise for the future. Furthermore, the development of inhibitors targeting NLRs and PGLYRPs offers potential in slowing the progression of the aforementioned diseases. Patients suffering from conditions like Crohn's disease could potentially benefit from the utilization of probiotics, paraprobiotics, or fecal microbiota transplantation. These therapeutic implications, whether utilizing or inhibiting peptidoglycan or its immune receptors, offer promising prospects for the future. However, it is essential to underscore that further research is necessary to carefully weigh the benefits against the risks and ensure a balanced approach to disease management

Layman’s summary

The human gut microbiota, a collection of various tiny organisms, mainly bacteria, in our digestive system, plays a crucial role in maintaining our overall health. Normally, it remains stable, resilient, and forms a mutually beneficial relationship with our body. It also has a significant impact on essential bodily functions, such as our metabolism, immune system, and the absorption of nutrients. One specific component of the gut microbiota, called peptidoglycan fragments, is key to preserving this harmonious relationship. When this balance is disrupted, it can lead to the development of various diseases. This review article aims to investigate the possible connection between peptidoglycan fragments and the occurrence of different diseases. It also explores potential ways to use peptidoglycan for therapeutic purposes. It has become clear that when the sensing of peptidoglycan is disturbed, especially through certain immune receptors like NOD1, NOD2, and peptidoglycan recognition proteins, it can contribute to the onset of several diseases, including Crohn's disease, neurodevelopmental disorders, and heart conditions. These disruptions also affect the development of colorectal cancer. Surprisingly, peptidoglycan fragments can have a dual role in cancer, both promoting and inhibiting its growth. For example, they can enhance the effectiveness of PD-L1 anti-tumor immunotherapy. As a result, there is potential in using peptidoglycan fragments as a therapeutic approach to enhance the effectiveness of cancer immunotherapy in the future. Additionally, the development of inhibitors that target peptidoglycan-sensing receptors offers promise in slowing down the progression of the diseases mentioned above. Patients with conditions like Crohn's disease may also benefit from the use of drugs or fecal microbiota transplantation. These therapeutic strategies, whether using or inhibiting peptidoglycan, offer hopeful prospects for the future. However, it's crucial to emphasize that further research is needed to carefully evaluate the advantages and disadvantages and ensure a balanced approach to managing these diseases.

1. The human gut microbiota

Humans are inhabited by different sets of microorganisms like viruses, yeasts, and—most importantly and abundantly—bacteria. These microorganisms engage in a variety of interactions with the human body, including mutualistic, commensalistic, and pathogenic ones. The collective term for these microorganisms and their interactions with the human body is the human microbiota [1]. The human body harbors microbiotas in various locations, including the lung, gut, oral cavity, skin, and vagina [2]. The human gut microbiota is the most crucial location for this literature review. The human gut microbiota possesses approximately 150 times more genetic information than the entire human genome consisting of an estimated amount of gut microorganism somewhere between 10^{13} and 10^{14} [3].

With usually six different phyla, of which *Firmicutes* and *Bacteroidetes* are the two main types, the human gut microbiota is thought to be the most crucial to maintaining our health [4]. Under normal circumstances, the gut microbiota demonstrates stability, resilience, and a symbiotic relationship with the host. High taxonomic diversity, microbial gene richness, and stability of the core microbiota are often indicators of a healthy microbiota community; however, these characteristics may differ from person to person due to differences in aging and environmental factors [4]. Diversity typically increases from childhood to adulthood and diminishes after the age of 70. A healthy gut microbiota plays a key role in metabolic, immune, and nutrient extraction processes [5]. Multiple mechanisms are used to influence these biological processes. For instance, the versatile metabolic genes within the human gut microbiota provide biochemical pathways and unique enzymes to extract energy and nutrients from food [6]. Moreover, the human gut microbiota is crucial for the biosynthesis of bioactive molecules like amino acids, lipids and vitamins. Lastly, the production of antimicrobial substances by the gut microbiota significantly contributes to the host's defense against external pathogens and plays a crucial role in the immune system and intestinal mucosa development [7]. The gut microbiota is important in maintaining symbiosis, and when this balance is disrupted, it is strongly associated with different kind of diseases such as inflammatory bowel disease (IBD). Notably, peptidoglycan fragments derived from the human gut microbiota play a significant role in maintaining this symbiotic relationship.

Since the microbiota's role in health and disease was discovered by numerous studies, it has received more and more attention. Extensive research has been conducted into the connections between the microbiota and various illnesses, including cancer and neurological disorders, while concurrently exploring the potential therapeutic applications of manipulating the human microbiota [7]. This literature review aims to provide a comprehensive overview of our current mechanistic understanding regarding the contribution of the human microbiota to the development of diverse diseases, such as Crohn’s disease, cardiovascular diseases (CVDs) and various neurodevelopmental disorders, as well as its clinical potential, particularly in the context of cancer treatment, for instance immunotherapy. The primary objective of this review is to investigate whether there exists a relationship between the emergence of diseases and the role of peptidoglycan fragments, one of the metabolites originating from the human microbiota. These fragments trigger the release of pro-inflammatory cytokines and the activation of T cells in response to induced inflammation [8]. In addition, this literature review will also explore the potential therapeutic applications of peptidoglycan fragments. A deeper understanding of the role of peptidoglycan within the human gut microbiota holds the promise of effective disease management through microbiota manipulation.

2. Peptidoglycan fragments derived from human gut microbiota

The function of peptidoglycan

Numerous Gram-positive and Gram-negative bacteria make up the human gut microbiota. Both of these bacteria's cell walls contain peptidoglycan, an essential and unique component of the bacterial cell wall crucial for structural integrity [8]. Gram-positive and Gram-negative bacteria differ in the composition of their cell wall peptidoglycan (**Figure 1A**). Gram-positive bacteria have a multi-layered peptidoglycan cell wall that forms the outside of the bacteria without an additional outer membrane, whereas Gram-negative bacteria have a thinner peptidoglycan layer surrounded by an outer membrane. The outer membrane of Gram-negative bacteria contains lipopolysaccharides (LPS), which is a distinctive feature unique to Gram-negative bacteria [9]. Peptidoglycan primarily functions to withstand turgor pressure and preserve cell integrity, while also playing a key role in cell division, growth, cell shape and protection against environmental challenges [10]. Inhibiting the biosynthesis of peptidoglycan, for example, through mutations, certain classes of antibiotics, or enzymatic degradation by enzymes such as lysozyme, results in cell lysis, confirming its vital role [11]. While the overall structure of peptidoglycan remains consistent across many organisms, variations between bacterial species arise from modifications in the backbone and peptide crosslinking. Peptidoglycan's fundamental structure consists of two backbones connected by a central stem peptide. Two repeating disaccharides, N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNac), are connected by β(1-4) linkages to form the peptidoglycan backbone. The fundamental structure of the central stem peptide consist of an amino acid sequence of L-Ala-γ-D-Glu/isoGln-X-D-Ala, where the third amino acid (X) typically corresponds to either meso-diaminopimelic acid (mDAP) or L-Lysine (Lys) [12]. Based on this common structure, there are two major types of peptidoglycan: Gram-positive bacteria predominantly feature Lys-type peptidoglycan in their cell walls, while Gram-negative bacteria and a subset of Gram-positive bacteria incorporate diaminopimelic acid (DAP)-type peptidoglycan into their cell walls (**Figure 1B**) [9].

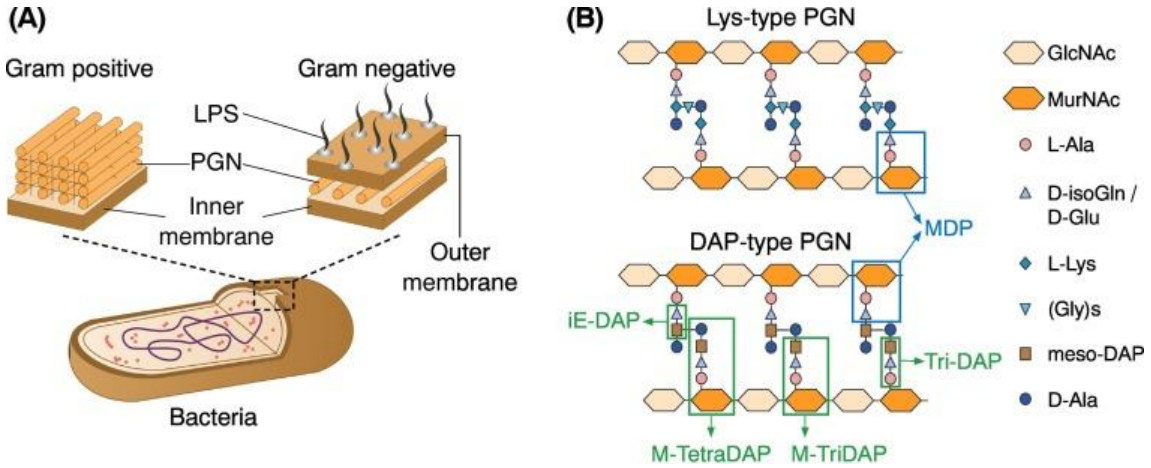

Figure 1: Schematic representation of bacterial peptidoglycan structure and location (A) *The distinctions in peptidoglycan composition between Gram-positive and Gram-negative bacteria is depicted here. Gram-positive bacteria feature a multi-layered peptidoglycan cell wall without an outer membrane, while in contrast, Gram-negative bacteria exhibit a comparatively thin peptidoglycan layer enveloped by an outer membrane. This outer membrane contains LPS, a feature absent in Gram-positive bacteria.* ***(B)*** *The peptidoglycan backbone is comprised of repeating disaccharides such as MurNac and GlcNAc linked by β(1-4) bonds. The central stem peptides form crosslinks between adjacent strands, typically composed of a mix of four L- and D-amino acids. Based on this structure, two primary types of peptidoglycan are observed: Lys-type peptidoglycan, predominantly found in Gram-positive bacteria, and DAP-type peptidoglycan, present in both a subset of Gram-positive bacteria and all Gram-negative bacteria. Specific motifs like MDP and Tri-DAP are recognized by PRRs such as the NOD-like receptors NOD1 and NOD2 (Adapted from* [9]*).*

Sugar polymer lengths vary between bacterial species, with constant reforming and degradation to support bacterial replication and growth [11]. In addition to varying the length of the polymer chains, bacterial species also use modifications of the sugar backbone or their amino acid side chains such as deacetylation, N-glycosylation, and O-acetylation. Post-synthetic modifications serve multiple purposes, including promoting growth and sporulation, and allowing pathogenic bacteria to evade the host's innate immune system [13]. This modifications, which can be considered as “remodeling” or “editing” of peptidoglycan, play a critical role in pathogenesis and enhance bacterial flexibility [8]. N-deacetylation of GlcNac, MurNac, or both, for example, is used by various bacteria, including *Streptococcus pneumoniae* and *Bacillus anthracis,* to reduce sensitivity to lysozymes and thereby prevent degradation, as well as decreasing the activation of inflammasomes triggered by peptidoglycan [10], [14].

In addition to peptidoglycan remodeling and editing, both Gram-positive and Gram-negative bacteria engage in the turnover and recycling of their cell walls. With each generation of cell growth and division, approximately 50% of the peptidoglycan in the cell wall is systematically broken down and recycled which is advantageous for resource recovery [15]. The substantial distinctions in peptidoglycan thickness and the absence of an outer membrane in Gram-positive bacteria result in a different mechanism of cell wall turnover and recycling between these two bacterial groups [16]. In Gram-negative bacteria, as cells grow, approximately half of the preexisting peptidoglycan is broken down by peptidoglycan-cleaving enzymes like endopeptidases and lytic transglucosylases. These enzymes release peptidoglycan fragments from the cell wall into the periplasm. Subsequently, various transporters facilitate the uptake of these fragments, enabling their utilization in diverse processes such as glycolysis or murein synthesis. In the case of *E. coli* bacteria, cell wall recycling is a crucial process. The primary turnover product, anhydromuropeptides (e.g., GlcNac-1,6-anh-MurNAc-tetrapeptide), generated from peptidoglycan, is taken up by various transporters, including the AmpG transporter. Subsequently, these products are further processed in the cytoplasm. From there, the turnover products are directed either towards peptidoglycan synthesis or into energy-generating pathways [16]. In numerous Gram-positive bacteria, counterparts of recycling enzymes have been identified, suggesting the presence of cell wall recycling in these bacteria. However, the extent of this turnover process varies considerably among Gram-positive bacteria. For instance, *E. faecalis* exhibits only minimal turnover. For most of these Gram-positive bacteria, studies have not yet uncovered the specific cell wall hydrolases or the regulatory mechanisms of lytic enzymes involved in this process [16].

In the course of regular peptidoglycan turnover and cell growth, small fragments of peptidoglycan are generated and released in various forms, including peptidoglycan monomers, dimers, and free peptide stems. These fragments are recognized for their capacity to induce fever (pyrogenic) and cause harm to cells (cytotoxic effects) [17]. One example is seen in the bacteria *N. gonorrhoeae*. When this bacterium releases peptidoglycan fragments, it triggers cell death in ciliated cells within an organ culture of the human Fallopian tube. This cell death occurs as a result of the activation of the innate immune system, leading to the production of proinflammatory cytokines, which subsequently prompts the recruitment of neutrophils [18].

Peptidoglycan activating the immune system

The human innate immune system plays a crucial role in distinguishing self from non-self and defending against pathogenic microbial products. One way it initiates inflammatory responses is by recognizing pathogen-associated molecular patterns (PAMPS) through multiple classes of pattern recognition receptors (PRRs) [19]. Peptidoglycan and peptidoglycan fragments serve as essential

PAMPS that interact with the innate immune system via numerous PRRs, expressed on cell surfaces, secreted, or found intracellularly, making peptidoglycan a vital player during bacterial infections.

PGLYRPs

Peptidoglycan recognition proteins (PGLYRPs: PGLYRP1-PGLYRP4) represent one group of these PRRs. PGLYRPs share a conserved C-terminal amidase domain comprising roughly 160 amino acid residues. This domain exhibits specific binding affinity for muramyl-penta, tri-, or tetrapeptides. Moreover, in the presence of zinc ions (Zn2+), PGLYRPs can engage with and cleave peptidoglycan fragments, employing amidase activity via either a catalytic or non-catalytic pathway. PGLYRP2 stands out among these receptors due to its amidase activity, playing a pro-inflammatory role in peptidoglycan-induced inflammation by hydrolyzing peptidoglycan. The other PGLYRPs primarily contribute to enhancing immune activation pathways or fulfilling pivotal roles in signal transduction. These non-catalytic PGLYRPs are typically found in extracellular, intracellular, or transmembrane locations [12]. PGLYRPs have the capacity to enhance the immune system through two distinct mechanisms. Firstly, they can detect the presence of bacteria with peptidoglycan and subsequently activate various signaling pathways. For instance, by recognizing monomeric or polymeric mDAP-peptidoglycan, they initiate the immune deficiency pathway which is a nuclear factor-κB (NF-κB) signaling pathway. This, in turn, triggers the production of antimicrobial peptides, further enhancing the body's defense against pathogens by producing a potent antibacterial defense response [12]. Secondly, PGLYRPs can also be activated by peptidoglycan found within bacterial cell walls. This activation leads to an antibacterial response by triggering a bacterial two-component system in both Gram-positive (CSSR-CSSS) and Gram-negative bacteria (CPXA-CPXRIN). Both these systems are capable of initiating bacterial cleavage and causing damage through multiple means, including membrane depolarization and oxidative processes, ultimately resulting in bactericidal effects [20]. Moreover, studies suggest that PGLYRP-mediated activation of stress and oxidative response systems induces bacterial suicide [21]. Furthermore, PGLYRPs cooperate with PRRs, enhancing phagocyte recognition of bacteria and aiding in bacterial elimination [22].

NOD-like receptors

Cytosolic NOD-like receptors (NLRs), NOD1 and NOD2 are well-defined sensors for peptidoglycans. These receptors are expressed in various cell types including epithelial cells and myeloid phagocytes [23]. The major receptor for peptidoglycan is NOD1 which is constitutively expressed at higher levels than NOD2 in many cell types and tissues [24]. NOD1 activation specifically targets Gram-negative bacteria, while NOD2's ligand is present in both Gram-positive and Gram-negative bacteria [25]. The difference in activation mechanisms can be attributed to variations in peptidoglycan motifs. For example, NOD2 is activated when it recognizes an intact MurNAc moiety, such as muramyl dipeptide (MDP) consisting of MurNAc linked to a dipeptide (L-Ala-γ-D-isoGln). This MDP component is prevalent in the cell walls of both Gram-positive and Gram-negative bacteria. Conversely, NOD1's activation relies on the presence of segments from the DAP-type peptidoglycan. These agonists of NOD1 can take diverse forms, including muramyl tripeptides (M-TriDAP) or tetrapeptides (M-TetraDAP). Moreover, ligands such as Tri-DAP (L-Ala-γ-D-Glu-mDAP) and iE-DAP (γ-D-Glu-mDAP) also prove effective in activating the NOD1 receptor. These specific agonists are predominantly encountered within DAP-type peptidoglycan, which is primarily found in Gram-negative bacteria and only in a subset of Gram-positive bacteria (see **Figure 1B**) [9].

For NOD1 and NOD2 sensors to function, they must either detect peptidoglycan fragments which are degraded and transported into the cytosol or detect bacteria present in the cytosol [26]. Activation of NOD1 and NOD2 by their respective ligands triggers two signaling pathways, involving the mitogen-

activated protein kinase (MAPK) signalling cascade and the NF-κB, leading to the production of chemokines and inflammatory cytokines [27]. Several transporters have been identified to facilitate the transport of peptidoglycan fragments into the cytosol. These transporters are identified under the SLC15 family, which are H+-coupled oligopeptide cotransporters. In dendritic cells and macrophages, two of these transporters, namely SLC15A3 and SLC15A4, are recognized for transporting the same ligands as NOD1 and NOD2. Knockout mice and dendritic cells lacking both SLC15A3 and SLC15A4 lose their sensitivity to NOD1 and NOD2 ligands. Furthermore, on the plasma membranes of epithelial cells, the transport of NOD2 ligands, mostly MDP, is facilitated by SLC14A1 (PEPT1) and SLC15A2 (PEPT2) [28]. Typically, the colon does not exhibit the expression of PEPT1 and PEPT2. Nevertheless, in cases of chronic colon inflammation, such as in IBD, there is an upregulation of these receptors. In this context, the transport of the Tri-DAP ligand exacerbates inflammation, as it can now enter colonocytes owing to the induced expression of PEPT1 and PEPT2 [29].

Alternatively, instead of the use of transporters to deliver NOD1- and NOD2-activating peptidoglycans into the cytosol, other systems such as pore-forming toxins and bacterial secretion systems have also been implicated in the delivery of peptidoglycan into the cells [27]. Phagocytic cells must internalize and degrade peptidoglycan and peptidoglycan-associated PAMPs to fully sense them. Numerous enzymes found in mammalian phagocyte lysosomes likely contribute to the breakdown of cell wall peptidoglycan. The enzyme lysozyme, a well-known example, specifically cleaves the β(1-4) linkages between MurNac and GlcNac. O-acetylation, one of the aforementioned modifications, makes peptidoglycan resistant to being degraded by lysozyme [11]. When peptidoglycan degrades in phagosomes, ligands are released, activating NOD2, TLR2, and NLRP3. Although the production of inflammatory cytokines such as IL-12 and TNF by immune cells like dendritic cells and macrophages is generally low upon activation, co-stimulation with TLR ligands, such as TLR9 stimulated by DNA, greatly enhances the overall response [30]. Additionally, the availability of other PAMPs is encouraged by the degradation of peptidoglycan, amplifying their signals and shaping a balanced immune response. However, bacteria capable of altering peptidoglycan's structure to resist lysozyme degradation can delay or diminish pro-inflammatory immune responses [8].

NLRP1 and NLRP3

Other NLRs, specifically NACHT, LRR, and PYD domains-containing protein 1 (NLRP1) and NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3), have been identified as cytosolic detectors of peptidoglycan [31]. These receptors are components of the inflammasome complex, which ultimately triggers the activation of the protease caspase 1 [8]. The activation of caspase 1 results in the production of pro-inflammatory cytokines, including IL-18 and IL-1β. Studies have revealed that, in certain scenarios, MDP, the activating ligand of NOD2, can also trigger NLRP3 and be detected by NLRP1 in conjunction with NOD2 [32]. Taken together, these observations highlight peptidoglycan's ability to trigger diverse innate immune sensing pathways, as stated in **Table 1**, underscoring its significant role in shaping the broader immune response against bacterial infections.

Table 1: Summary of the immune receptors involved in peptidoglycan recognition. *The table provides an overview of the peptidoglycan immune receptors along with their corresponding ligands and the resulting functions they trigger.*

Receptor(s)	Ligand(s)	Function(s)
PGLYRPs		
• PGLYRP1-4	• Both monomeric and polymeric mDAP-peptidoglycan • Peptidoglycan in bacterial cell wall	• Activate immune deficiency pathway via NF-κB • Activate antibacterial response via two-component system
NLRs		

• NOD1
• NOD2
	• NOD1: Segments of DAP-type peptidoglycan
	  ○ M-TriDAP
	  ○ M-TetraDAP
	  ○ Tri-DAP
	  ○ iE-DAP
	• NOD2: Intact MurNAc moiety
	  ○ MDP
		• Activate the MAPK signalling cascade
		• Activate the NF-κB signaling pathway
		  ○ Both leading to the production of pro-inflammatory cytokines and chemokines

SLC15 family		
• SLCA15A3 • SLCA15A4 • SLC14A1 (PEPT1) • SLC15A2 (PEPT2)	• Same ligands as NOD1 and NOD2, mostly MDP	• Transporting ligands into the cytosol
Other NLRs		

• NLRP1
• NLRP3
	• MDP
	  ○ Activation of NLRP3
	  ○ Detection by NLRP1 with NOD2
		• Activation of protein caspase 1 leading to production of pro-inflammatory cytokines

Peptidoglycan and systemic homeostasis in the gut

Detecting peptidoglycan in the intestines plays a vital role in maintaining microbiota and a healthy gut architecture. In both humans and mice, peptidoglycan recognition by NOD2 has an influence on the intestinal microbiota [33]. An example of this is the high levels of NOD2 expression in the Paneth cells within the intestines where it regulates the gut microbiota by producing antimicrobial proteins and peptides like α-defensins. Reductions in α-defensin levels have been observed to alter the gut microbiota in patients with Crohn's disease [34]. Additionally, peptidoglycan recognition by NOD1 is involved in shaping the microbiota composition and maintain gut homeostasis by promoting the formation of intestinal lymphoid tissues in mice. The formation of these tissues in the intestines relies on the sensing of bacteria, a process linked to NOD1. Furthermore, it has been reported that peptidoglycan from the gut can impact the functioning and development of myeloid cells throughout the body. Mice lacking NOD1 exhibit fewer neutrophils and inflammatory monocytes [35].

Not only are NLRs crucial for regulating the intestinal microbiota, but also peptidoglycan recognition by PGLYRPs play an important role. PGLRYPs are produced by the gut epithelial cells in order to maintain a healthy gut microbiome. Knockout mice lacking PGLYRP2 and PGLYRP3 are more susceptible to colitis, and variants of these receptors have been associated with an increased risk of Parkinsons disease in humans [36], [37]. Furthermore, in response to peptidoglycan fragments from the gut, PGLYRPs may also function in the brain. Mice raised in germ-free environments, compared to those with normal gut microbiota, exhibit increased motor activity and reduced anxiety-like behavior

[38]. This implies that the recognition of peptidoglycan by various immune receptors is linked to the onset of diverse diseases and disorders in both animals and humans This underscores its significance as a crucial component in preserving systemic homeostasis in the gut.

3. Linking microbiota-derived peptidoglycan to diseases

As previously mentioned, the recognition of peptidoglycan by NLRs and PGLYRPs play a crucial role in maintaining host homeostasis. Dysregulation in peptidoglycan signaling pathways has been linked to various diseases, including Crohn's disease, neurodevelopmental disorders such as Parkinson's disease, and CVDs. The diverse nature of these diseases makes it challenging to pinpoint a singular common cause. Nonetheless, a common thread among them is their connection to immune receptors and the associated signaling pathways, which will be explored in detail in this chapter.

Crohn's disease

Crohn's disease is a progressive gastrointestinal disorder characterized by chronic inflammation and bowel damage. The symptoms of this chronic inflammatory disease evolve in a remitting and relapsing manner [39]. About 70 genetic risk loci for Crohn's disease have been found by genome-wide association studies. The CARD15 gene encoding NOD2 is responsible for the majority of Crohn's disease polymorphisms. This is primarily due to the fact that mutations in the gene frequently result in impaired NOD2 sensing [40]. According to research, the strongest risk factor for this illness is specifically loss-of-function mutations in NOD2, causing ligand recognition impairment and accounting for 50% of familial Crohn's disease cases in western populations [41]. However, it remains still elusive how peptidoglycan fragments in the gut can influence the NOD2 signaling and host pathology. Disruptions in peptidoglycan hydrolase activity and a decrease in available NOD2 ligands may result from changes in the gut microbiota composition, contributing to the development of Crohn's disease [42].

DL-endopeptidases, derived from *Firmicutes*, are essential peptidoglycan hydrolases involved in NOD2 ligand production. Research has shown that intact peptidoglycan can be degraded into NOD2 ligands when combined with muramidase and DL-endopeptidase [43]. Furthermore, studies have indicated that DL-endopeptidase-encoding genes exhibit variations in their presence and abundance, while muramidase may play a redundant role in the gut microbiome. It has been observed that DL-endopeptidases genes are universally decreased in Crohn's disease patients and negatively correlated with colitis activity. Moreover, mice are more likely to develop colitis when exposed to the fecal microbiota of Crohn's disease patients with low DL-endopeptidase activity while on the contrary treatments based on DL-endopeptidase, such as administration of the *L. salivarius* strain that produces DL-endopeptidase, effectively reduced colitis via NOD2. These findings highlight that, in addition to genetic mutations, gut microbiota dysbiosis can diminish NOD2 signaling due to a lower concentration of DL-endopeptidases and NOD2 ligands in the gut. This exacerbates Crohn's disease pathogenesis by failing to activate NOD2, even when the NOD2 itself remains intact, resulting in the loss of its regulatory capacity in the immune system [42]. Another study reported the presence of a bifunctional peptidoglycan hydrolase (LPH) with both DL-endopeptidase and muramidase activity. High-efficiency MDP production by this enzyme triggers NOD2 activation and thereby maintaining gut homeostasis while exerting anti-colitis effects through NOD2 signaling [44]. Together, these studies emphasize the role of secreting peptidoglycan hydrolases into the gut in manipulating the murapeptides-NOD2 pathways, which is essential for maintaining gut homeostasis or potentially developing Crohn's disease.

Neurodevelopmental disorders

The gut-brain axis, a highly sophisticated bidirectional communication network between the gut and the central nervous system, has long been recognized. The gut microbiota has recently come to be understood as an essential "third component" of the gut-brain axis, giving rise to the new idea of the

microbiota-gut-brain axis [9]. Communication between the brain and the microbiota occurs through various mechanisms, one of which involves microbial metabolites like peptidoglycans. Peptidoglycans can migrate from the gut microbiota's mucosa to the systemic circulation under physiological conditions, where they interact with bone marrow-derived neutrophils [9]. Notably, these neutrophils are typically not found in the human gut, indicating the presence of peptidoglycan fragments beyond the gut. The influence of peptidoglycan fragments from the human gut microbiota on brain functions is not a novel discovery. Previous research has already demonstrated the presence of peptidoglycan fragments in the cerebrospinal fluid and brains of sleep-deprived animals, suggesting a potential role for peptidoglycan in the development of sleep disorders. Furthermore, the developing brain of postnatal mice exhibits high expression levels of NLRs, PGLYRPs, and the peptidoglycan transporter PEPT1 during specific developmental stages, indicating that peptidoglycan fragments may have varying effects on brain development depending on the postnatal age [9].

Studies have demonstrated the presence of peptidoglycan fragments and their receptor NOD1 in the brains of normally developing mice, where they serve as key regulators of systemic effects. This suggests that, under normal circumstances, peptidoglycan can traverse the blood-brain barrier [45]. In addition to NOD1, PGLYRP2 was found to be highly expressed in neurons in several brain regions including the cerebellum, prefrontal cortex and hippocampus, implying that peptidoglycan can directly influence neurons [46]. Notably, aged mice transgenic for the human peptidoglycan sensing molecule PGLYRP2 exhibited significant sex-dependent changes in motor and anxiety-like behaviors. In this instance, female mice with PGLYRP2 deficiency perform better motorically than male mice. They do, however, exhibit higher levels of anxiety-like behavior, indicating that PGLYRP2's modulatory effects on the brain depend heavily on a variety of host factors, including age, sex, and the type of neuronal circuit. Furthermore, alterations in synaptic-related gene expression were subtly observed in brain regions associated with the processing of emotional stimuli [47]. The peptidoglycan transporters, as well as NOD1, and NOD2, are all highly expressed in the human placenta meaning that peptidoglycan can cross the placental barrier and affect the developing brain. Disrupting this signaling pathway's elements may result in abnormal motor, social, and cognitive development [48]. During particular windows of postnatal brain development, both the PGLYRPs and NLRs are expressed. However there are some sex-dependent and brain-region dependent differences observed in the expression in the brain of peptidoglycan-sensing molecules. Specifically, NLRs and PGLRYP1 are more expressed in males while PGLRYP2, PGLRYP3 and PGLRYP4 are higher expressed in females. Notably, this differences were more observed in the prefrontal cortex, a crucial area involved in a number of psychical and neurodevelopmental disorders such as autism spectrum disorder [49]. Altogether, this data suggest a role of peptidoglycan in brain and neurodevelopmental disorders.

One example of this can be observed in Parkinson's disease, which is recognized as a systemic disorder in which the gut microbiota may potentially play a role. The risk of developing Parkinson's disease has been notably linked to genetic mutations in three of the four PGLYRPs (2,3 and 4) [50]. Additionally, various enzymes such as nitric oxide synthase and cyclooxygenase, both known to be involved in the pathogenesis of Parkinson's disease, as well as inflammatory cytokines, have been found to be upregulated in a peptidoglycan model of Parkinson's [50]. It's important to note that these findings are reported by a single study, and further investigation is required to establish a concrete connection between Parkinson's disease and peptidoglycan fragments. Another example can be seen in multiple sclerosis (MS), a chronic inflammatory disease characterized by severe impairment of the blood-brain barrier integrity in lesions with inflammatory activity. Essentially, peptidoglycan fragments can directly access these lesion areas. Peptidoglycan has been identified in non-human primate models of MS and in phagocytes within demyelinating lesions from MS patients [51]. The presence of peptidoglycan was

also evaluated in resected brain tissues from 30 live patients in a 2019 deep sequencing study, including two specimens taken at various points from a single MS patient. This study revealed that several brain samples from MS patients contained peptidoglycan, which could potentially contribute to local neuroinflammation within demyelinating lesions, further exacerbating demyelination [52]. In MS animal models, collectively referred to as experimental autoimmune encephalomyelitis (EAE), it has been demonstrated that peptidoglycan-neutralizing monoclonal antibodies can limit mouse EAE, suggesting that endogenous peptidoglycan may play a role in brain inflammation [53]. Both of these results imply that the presence of peptidoglycan fragments in the brain could potentially interfere with normal synaptic functions [51].

Cardiovascular diseases

In addition to its association with Crohn's disease, an imbalanced gut microbiota can also contribute to CVDs, including heart failure and coronary artery disease, which account for 31% of global deaths. Atherosclerosis, responsible for roughly 50% of CVD-related deaths, is a major risk factor for these diseases [54]. Peptidoglycan has garnered significant attention in relation to CVD risk. Numerous studies have linked intestinal dysbiosis to alterations in the intestinal epithelial barrier. Reduced expression and reorganization of tight junction proteins, as well as an imbalance between intestinal epithelial cell death and proliferation can result in intestinal permeability. To prevent the translocation of intestinal contents, including peptidoglycan fragments, into the bloodstream, maintaining proper barrier function is crucial. Peptidoglycan has been implicated in the development of atherosclerosis when fragments of it leak into the bloodstream, activating immune cells in the circulation [55]. Notably, peptidoglycan has been detected in human atherosclerotic plaques and has been associated with increased plaque inflammation [56]. Additionally, lower levels of antibodies against peptidoglycan has been measured in human atherosclerotic plaques [57]. In the pathological process of atherosclerosis, a study demonstrated that PGLYRP1 may be one of the key regulators of endothelial dysfunction. Patients with coronary artery disease have shown higher serum PGLYRP1 concentrations compared to individuals with healthy coronary arteries. Furthermore, PGLYRP1 expression was found to be increased in murine atherosclerotic plaques [57], [58]. Elevated levels of PGLYRP1 have also been independently associated with a higher risk of initial atherosclerotic cardiovascular events, implying that the biological processes of elevated PGLYRP1 are related to the development of clinical atherosclerotic CVD [59]. Furthermore, metagenomic sequencing has revealed that individuals with stenotic carotid artery plaques exhibit an enrichment of genes related to peptidoglycan production in their gut microbiome, whereas control groups show enrichment in genes responsible for synthesizing antioxidants and anti-inflammatory molecules. This suggests that increased peptidoglycan production by the gut metagenome may contribute to atherosclerosis symptoms by activating neutrophil function and the innate immune system [60]. Nevertheless, the reason for the heightened presence of peptidoglycan production genes remains unclear, as it cannot be attributed to either Gram-positive or Gram-negative bacteria, given that both types possess peptidoglycan. However, it is noteworthy that the control group exhibited an enrichment of Gram-positive bacteria [60].

4. Potential therapeutic approaches using microbiota-derived peptidoglycan

As previously noted, diseases can arise from impaired signaling of immune receptors associated with peptidoglycan binding. Despite the adverse effects of peptidoglycan on these diseases, it is noteworthy that peptidoglycan derived from the human gut microbiome also holds therapeutic potential. For instance, it could improve the efficacy of treatments like PD-L1 immunotherapy for cancer patients. Furthermore, this chapter will explore the potential of manipulating the gut microbiota to prevent the onset and/or progression of the mentioned diseases.

Promoting cancer immunotherapy

Cancer immunotherapy has proven to be clinically effective in addressing diverse hematological and solid tumors by utilizing the patient's immune system to obstruct tumor growth. Immune checkpoint inhibitor proteins such as PD-1, PD-L1 and CTLA4 are targeted by antibodies to treat a number of human cancers although the patients' responses vary considerably [61]. The differences observed among patients can be attributed to a multitude of factors, including variations in lymphocyte recruitment and infiltration, the mutational burden of the malignancy, signaling cues within the tumor microenvironment, and the effectiveness of tumor antigen presentation [62]. Furthermore, a newly identified influential factor affecting the effectiveness of cancer immunotherapy, including anti-PD-L1 treatment, is the gut microbiota [63]. This factor has been observed in multiple human and animal study groups, where the responsiveness to cancer immunotherapies is linked to the presence of particular microbial species [62]. In human cohorts of patients undergoing anti-PD1 immunotherapy, microbiome analyses have revealed that responsive patients exhibit an enrichment of the bacterial genus *Enterococcus* [64]. These strains of *Enterococcus* bacteria are recognized for their diverse functions in regulating infections, graft-versus-host disease, autoimmunity, and their ability to activate immune signaling pathways. Additionally, commensal strains of the bacteria *E. faecium* and *E. faecalis* are commonly utilized as probiotics in both human and animal contexts [65]. Consequently, it becomes conceivable that distinct *Enterococcus* species may have the potential to enhance responses to cancer immunotherapy. Nonetheless, other bacterial strains, including *Bifidobacterium bifidum* and *Lactobacillus*, have demonstrated enrichment in peptidoglycan biosynthetic genes, thereby exhibiting synergy with PD-1 blockade [62].

In a study involving B-16-F10 melanoma-positive mice, the supplementation of both high and low doses of the human commensal bacteria *E. faecium* together with anti-PD-L1 immunotherapy resulted in a significant reduction in tumor size when compared to treatment with anti-PD-L1 alone (**Figure 2A**) [62]. Nevertheless, further analysis of the human gut microbiota in responsive patients revealed that additional *Enterococcus* species, namely *E. durans, E. hirae,* and *E. mundtii*, exhibited antitumor activity [64]. Notably, all four of these immunotherapy-active *Enterococcus* bacteria were found to be interconnected due to their unique peptidoglycan composition and remodeling capabilities, which enhance the host's tolerance to enteric pathogens. These capabilities are facilitated by conserved amidases and peptidases, and it was discovered that among these four active *Enterococci*, the NlpC/p60 hydrolases were remarkably conserved. These hydrolases include the peptidoglycan hydrolase known as secreted antigen A (SagA), which plays a role in breaking down components of the bacterial cell wall, leading to the release of muramyl peptide fragments such as GlcNAc-MDP [66]. These fragments originating from peptidoglycan serve as stimulatory molecules by binding to the NOD2 receptor, consequently enhancing the activation of the innate immune system and improving the responses to immunotherapy by increased activation of NF-κB and consequently the production of proinflammatory cytokines such as IL-1β (**Figure 2B**) [62]. The presence of bacteria expressing the SagA peptidoglycan hydrolase not only enhanced the effectiveness of anti-PD-L1 treatment in B16-F10

melanoma-positive mice but also resulted in a noteworthy reduction in tumor growth when mice with MCA205 fibrosarcoma or MC38 colorectal carcinoma cells were co-administered with SagA-expressing *Enterococci* and either anti-PD-1 or anti-CTLA4 immunotherapy, respectively. In all mice colonized with *E. faecalis*-SagA, there was a notable overall increase in the absolute number of CD3+ lymphocytes at the site of the tumor, with a particular emphasis on the rise in CD8+ T cells [62]. This data suggests that *Enterococci* possessing unique NlpC/p60 peptidoglycan hydrolase activity are capable of generating NOD2-active muropeptides and can influence the effectiveness of checkpoint blockade immunotherapy in live animal models by activating macrophages directly near the tumor for tumor cell clearance [62]. Therefore, the presence of NOD-2 active muropeptides and NlpC/p60 hydrolases can serve as valuable indicators for predicting therapeutic responses, making them clinically relevant biomarkers. On top of that, the composition of the microbiota in the human gut can be examined as a functional indicator of the therapeutic efficacy of cancer immunotherapies and may hold clinical relevance in predicting patients' therapeutic responses. Given these promising results in improving cancer immunotherapy, peptidoglycan fragments or other synthetic small molecules that activate peptidoglycan PRRs could serve as enhancers in PD-1, PD-L1, CTLA4, and potentially other immunotherapies, amplifying patient responses.

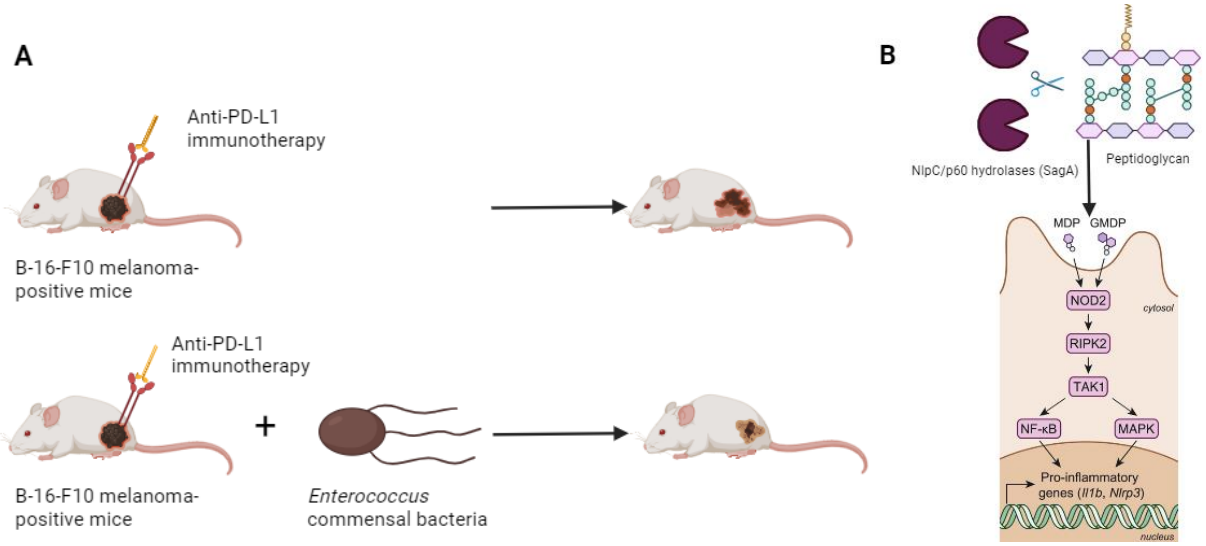

Figure 2: Mechanistic overview of simultaneous supplementation of both commensal* Enterococcus *bacteria and anti-PD-L1 immunotherapy in B-16-F10 melanoma-positive mice (A) *Mice treated only with anti-PD-L1 immunotherapy exhibited a noticeably less significant reduction compared to those subjected to a combined treatment involving both anti-PD-L1 immunotherapy and commensal Enterococcus bacteria, including E. faecium, E. durans, E. hirae, and E. mundtii. These four immunotherapy-active Enterococcus bacteria exhibited antitumor activity attributed to their unique peptidoglycan composition and remodeling capabilities both enhancing their host tolerance to enteric pathogens.* ***(B)*** *The NlpC/P60 hydrolases, particularly the peptidoglycan hydrolase SagA, exhibited the highest level of conservation among these Enterococcus bacteria. These hydrolases play a crucial role in breaking down peptidoglycan components into muramyl peptides, such as MDP and GlcNAc-MDP (GMDP). Both of these peptides serve as ligands activating the NOD2 receptor, leading to the transcription of pro-inflammatory genes and subsequently triggering the production of pro-inflammatory cytokines, including IL-1β. This cascade is a result of increased activation of the MAPK and NF-κB signaling pathways (made in BioRender.com and adapted from* [62]).

Nevertheless, in a study involving patients with B cell lymphoma who underwent chimeric antigen receptor (CAR)-T cell therapy, contrasting results emerged [67]. In this particular study, patients were administered broad-spectrum antibiotics prior to receiving CD19-targeted CAR-T cell therapy. Consequently, an increase in microbial pathways associated with peptidoglycan synthesis was detected. This phenomenon correlated with an elevated rate of disease progression and a decreased

overall survival rate [67]. Notably, the peptidoglycan biosynthesis pathway exhibited a higher presence in the gut microbiomes of patients who did not survive the initial six months following the therapy infusion [67]. This suggests that the role of peptidoglycan remains controversial, as it enhances the efficacy of cancer immunotherapies such as PD-1, PD-L1, and CTLA4, while simultaneously reducing the overall survival rate of patients undergoing CAR-T cell therapy.

Inhibiting tumorigenesis

Peptidoglycan is not only capable of amplifying the impact of cancer immunotherapy, but previous research has also highlighted its role in possessing anti-tumor effects in humans. It possesses the ability to inhibit tumorigenesis and halt the advancement of cancer. An example of this phenomenon is seen in colorectal cancer (CRC), a highly lethal and prevalent form of cancer [68]. Globally, nearly 700,000 individuals die to CRC, making it the second most significant contributor to cancer-related fatalities worldwide. A substantial 70% of these cases are influenced by environmental factors, with a notable role played by the gut microecology [69]. Within CRC, the commensal bacteria of the gut microbiota serve a double function by either promoting or inhibiting cancer.

Numerous studies, utilizing human cancer cell lines or cells, have provided evidence that the gut microbiota can inhibit CRC by triggering apoptosis or constraining cell proliferation due to different gut microbiota metabolites, such as short chain fatty acids, or components of gut microbiota such as peptidoglycan [68]. In a study conducted on a human colon cancer HT-29 cell line, peptidoglycan derived from a *Lactobacillus* strain exhibited significant anticancer effects [70]. These effects were primarily characterized by the induction of apoptosis and the inhibition of HT-29 cell proliferation. To confirm that these effects were specific to cancer cells, a noncancerous cell line was used, and in this case, peptidoglycan demonstrated only minimal toxic activity [70]. Further investigations were conducted to confirm the induction of apoptosis. Up and down regulation of apoptotic genes was measured, revealing that peptidoglycan upregulated proapoptotic genes while downregulating antiapoptotic genes. Additionally, an increase in the release of cytochrome-C from the mitochondria into the cytosol was observed. This is a crucial component of the signaling pathway that leads to enhanced apoptosis, ultimately activating killer proteases and resulting in the killing of the cells [70]. Considering the promising results in inhibiting CRC cells in vitro, there is growing anticipation that peptidoglycan could potentially emerge as a viable treatment for CRC in the near future.

As mentioned earlier, commensal bacteria play a dual role, with the potential to promote rather than inhibit tumorigenesis. One mechanism by which this occurs is through microbial factors like peptidoglycan, which can activate signaling pathways or foster tolerance. These actions can result in chronic inflammation, a significant risk factor associated with CRC [71]. The risk of developing CRC is elevated in patients with IBD [72]. Inflammation-associated cancer involves various influential factors, among them NF-κB and TLRs, driven by exposure to gut-residing microbes. One significant mechanism that triggers cellular changes contributing to the promotion or initiation of carcinogenesis is the activation of the TLR pathway through microbial outer membrane vesicles (OMVs). These vesicles can transport microbial components, including peptidoglycan, as cargo [73]. In this case, peptidoglycan can activate NOD or TLR signaling, for instance TLR2, leading to the production of cytokines and the recruitment of leukocytes. Nevertheless, disruptions in the microbial community can disrupt the balance, potentially overwhelming the immune system and thus fostering chronic inflammation in the gut [71]. An illustrative example of this phenomenon is the bacterium *Porphyromonas gingivalis*, whose OMVs containing peptidoglycan induce a robust TLR2 response. This response can lead to the initiation of chronic inflammatory reactions, thus contributing to CRC [74]. Furthermore, peptidoglycan can traverse the intestinal epithelial cell layers via TLR2-dependent phagocytosis, multivesicular bodies

and exosome secretion, and subsequently interact with macrophages, stimulating the production of IL-6. This, in turn, exacerbates inflammation in CRC [75]. Altogether, this demonstrates that peptidoglycan has a dual role - it can inhibit tumorigenesis directly by promoting apoptosis but also, indirectly, contribute to CRC by stimulating chronic inflammation.

Manipulating the gut microbiota

As mentioned above, peptidoglycan and its receptors shows promise in fighting CRC and, notably, in other cancers linked to inflammation. Therefore, in the future, various strategies can be explored to manipulate the human gut microbiota or harness peptidoglycan as a potential therapeutic tool for a range of diseases, including CRC.

A potential approach to treating CRC and other diseases involves the use of probiotics, which are live microorganisms that, when administered in appropriate amounts, offer health benefits. They can be beneficial in two main ways: first, by suppressing the growth of harmful pathogens, and second, by maintaining intestinal balance through regulation of the host's immune system [76]. A relevant study involved *Clostridium butyricum*, which produced cell-free supernatants with antagonistic effects are used against enterotoxigenic *Bacteroides fragilis*, a major contributor to severe inflammatory diseases and CRC [77]. This strain of *Clostridium* could also show promise in inhibiting for example *Porphyromonas gingivalis*, a bacterium known for its robust TLR2 response via peptidoglycan, which can trigger chronic inflammatory reactions linked to CRC. Therefore, *C. butyricum's* cell-free supernatant holds potential as a biotherapeutic agent for preventing and treating IBD and/or CRC caused by peptidoglycan from *Porphyromonas gingivalis.* In addition to using probiotics, non-viable paraprobiotics or postbiotics present another potential therapeutic option for conditions like CRC and other diseases. Postbiotics are substances secreted by probiotics into their cell-free supernatants, and they comprise a mixture of metabolic products. On the other hand, paraprobiotics consist of inactivated microbial cells from probiotics, including both ruptured and intact cell components like peptidoglycans [78]. Peptidoglycans derived from probiotic bacteria have demonstrated anti-cancer properties in both *in vitro* and *in vivo* studies [78]. In a mouse model with colitis, the application of peptidoglycan derived from probiotic bacteria exhibited anti-inflammatory effects. Additionally, the T-cell response was enhanced in immunocompromised, malnourished mice when treated with peptidoglycan from the probiotic bacteria *L. rhamnosus*. Furthermore, when various cancer cell lines were exposed to peptidoglycan derived from *L. casei*, it showed promising anti-tumor effects in vitro. In summary, this data suggests that the utilization of probiotics, paraprobiotics, and/or postbiotics containing peptidoglycan holds significant potential for enhancing anti-tumor effects.

Furthermore, an alternative therapeutic approach could involve fecal microbiota transplantation. As previously discussed, peptidoglycan fragments have been implicated in the onset of various diseases, including Crohn's disease, neurodevelopmental disorders, and CVDs, all of which are associated with gut microbiota dysbiosis. Fecal microbiota transplantation may serve as a valuable method for restoring this microbial balance. This was illustrated in a separate study involving rats with autism spectrum disorders, a group of neurodevelopmental conditions, where fecal microbiota transplantation led to a reduction in peptidoglycan biosynthesis thereby restoring the dysbiosis in the gut microbiota [79]. This highlights that fecal microbiota transplantation, with its capacity to restore gut microbiota dysbiosis, represents a promising and innovative treatment option for various diseases. Both probiotic treatment and fecal microbiota transplantation have demonstrated their potential, particularly in hepatitis B and hepatitis C virus infections, where the introduced bacteria act as valuable supplements with antibacterial and antiviral properties against these viruses [80]. This suggests that both techniques hold promise for addressing diseases linked to peptidoglycan fragments as well.

Furthermore, screening patients for the presence of peptidoglycan hydrolases or free MDP could serve as a valuable biomarker in the mentioned diseases. Elevated concentrations can activate various immune receptors, contributing to the maintenance of gut homeostasis. Conversely, lower concentrations may indicate a higher risk for the development of the aforementioned diseases.

Discussion

This literature review highlights the pivotal role played by peptidoglycan fragments in maintaining the balance of the human gut microbiota, crucial for overall homeostasis. Disruptions in this equilibrium can contribute to a range of diseases, including Crohn's disease, neurodevelopmental disorders, and CVDs. These disruptions often originate from impaired peptidoglycan sensing, particularly by receptors such as NLRs and PGLYRPs, as summarized in **Table 2**. Moreover, peptidoglycan fragments are implicated in the progression of various cancer types, with a notable impact on CRC. However, it's important to note that these fragments can play a dual role: not only promoting tumorigenesis but also inhibiting it. For instance, they may enhance the effectiveness of PD-L1 anti-tumor immunotherapy. The promising results in cancer immunotherapy suggest that peptidoglycan fragments could serve as enhancers, potentially enhancing patient responses. Additionally, their utility extends to the realm of probiotics, paraprobiotics, or fecal microbiota transplantation, offering opportunities to enhance outcomes for patients dealing with various diseases.

Table 2: Summary of peptidoglycan-associated diseases mentioned in this literature review. *This table provides an overview of diseases associated with peptidoglycan, along with any known receptors involved in the sensing process and the potential effects of peptidoglycan sensing.*

Disease(s)	Receptor(s)	Effect(s)
Inflammatory bowel diseases		
• Crohn's disease	• NOD2 (via gene mutations or ligands e.g. Muramyl dipeptide)	• Development of disease
Neurodevelopmental disorders		
• Parkinson's disease • Multiple sclerosis	• PGLYRP2,3,4 (via gene mutations) • Not known	• Development of disease • Local neuroinflammation, further exacerbating demyelination
Cardiovascular diseases		
• Atherosclerosis	• PGLYRP1	• Increased risk of first atherosclerotic cardiovascular disease
Cancer		
• Colorectal cancer • "Immunotherapy	• TLR2 • NOD2	**Dual role:** • Inhibiting of disease: triggering apoptosis or constraining cell proliferation • Promoting of disease: fostering chronic inflammation • Enhancing PD-1, PD-L1 and CTLA-4 immunotherapy

In addition to the diseases highlighted in the table above, there are other conditions in which peptidoglycan and its receptors play a significant role. While these diseases may be less extensively studied, they are worth mentioning. One such example is non-alcoholic fatty liver disease, in which bacterial products, including peptidoglycan, are elevated and can translocate from the gut into the portal circulation and then to the liver. A study has shown that peptidoglycan can induce inflammation and liver steatosis independently by activating the NOD2 receptor in mice. This activation of NOD2 by peptidoglycan stimulates lipogenesis, ultimately leading to lipid accumulation, thereby contributing to steatosis and, in later stages, steatohepatitis [81]. As already mentioned, this is a relatively understudied area, and it is worth noting that this study is currently the only one to establish a connection between non-alcoholic steatohepatitis and peptidoglycan. Additionally, the recognition of

peptidoglycan by the NOD2 receptor has been suggested to pose a risk for metabolic diseases. Studies have reported that the deletion of NOD2 may lead to insulin resistance and, subsequently, diabetes and obesity in mice [82]. Normal peptidoglycan sensing by the NOD2 system is crucial for preventing bacteria from colonizing metabolic tissues through translocation from the gut. However, further research is essential to precisely understand the mechanisms through which NOD2's regular peptidoglycan sensing prevents the onset of insulin resistance.

Peptidoglycan is not the only microbiota-derived substance implicated in the pathogenesis of diverse diseases. Another such agent, LPS, glycolipids primarily originating from the surface of Gram-negative bacteria, has been recognized for its capacity to induce acute inflammatory responses through its interaction with the innate immune system. One prime example of a disease linked to LPS is IBD which is associated with a higher concentration of LPS. In this case, LPS exacerbates inflammation, while IBD amplifies endotoxemia and intestinal permeability, a combination that holds significant importance in the development of IBD [83]. The way in which this inflammation occurs is via the activation of a peptidoglycan receptor, specifically NLRP3, resulting in the production of proinflammatory cytokines, including TNF-α [83]. The significance of proinflammatory cytokines extends beyond their role in the initiation of IBD. The activation of signaling pathways by LPS and the consequent production of pro-inflammatory cytokines also play a contributory role in the pathogenesis of Alzheimer's disease [84]. Another example of microbiota-related compounds causing diseases involves a substance called bacterial adenosine diphosphate-heptose (ADP-Hep). This compound is found in both certain Gram-positive and most Gram-negative bacteria. In a study, it was revealed that a bacterium called *F. nucleatum* is abundant in cases of CRC. In this context, ADP-Hep derived from *F. nucleatum* activates ALPK1 on CRC cells, which in turn enhances the NF-κB pathway. This results in increased regulation of ICAM1, a glycoprotein involved in direct cell-to-cell interactions. The upregulation of ICAM1 leads to greater metastasis and cell adhesion between CRC cells and endothelial cells [85]. This highlights ADP-Hep as a pro-metastatic substance, operating differently from peptidoglycan in promoting disease progression.

A question that remains is whether the utilization of peptidoglycan fragments offers more advantages than employing inhibitors against peptidoglycan recognizing receptors. When it comes to immunotherapy, it is evident that using peptidoglycan fragments can enhance the therapeutic effect and potentially serve as adjuvants. However, as revealed in this literature review, an increase in peptidoglycan fragments can also exacerbate the progression of other diseases. The use of such adjuvants may elevate the risk of developing conditions like Crohn's disease or CVDs. Therefore, further research is needed to ascertain the safety and potential risks associated with the use of peptidoglycan adjuvants in immunotherapy, such as anti-PD-L1, to ensure that they do not inadvertently contribute to the development of other diseases. Furthermore, on the contrary, the development of inhibitors targeting NLRs, NOD1 and NOD2, along with PGLYRPs (PGLRYP1-4), holds promise for restraining the progression of several of the aforementioned diseases. However, it's crucial to recognize that both types of receptors play essential roles in enhancing immune responses against bacterial infections, each in its unique way. Inhibiting these receptors may introduce the possibility of unintended side effects in the form of increased susceptibility to bacterial infections. Consequently, further research is imperative to comprehensively explore the potential for therapeutically modulating or blocking the NOD1 and NOD2 receptors, as well as their associated signaling pathways, in order to weigh the benefits against the risks and ensure a balanced approach to disease management.

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