Name: Ron Huis in ‘t Veld
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Master: Infection and Immunity
Department: Medical Microbiology
Supervisor: Dr. K. van Kessel

Peptidoglycan: Structures and Recognition

Abstract

Bacteria are identified in many different shapes, like cocci and rods, and internal volumes from 10^{-2} to 10^{6} μm. For this different shapes and sizes, it sounds obvious that there is a regulated process that allows the cell wall of bacteria to grow and divide. The major structural component of the cell wall of bacteria is peptidoglycan (PGN). Peptidoglycan comes in two forms in bacteria. Gram-positive bacteria contain a thick layer of PGN outside the cell membrane and gram-negative bacteria have a thin layer f PGN between their inner and outer cell membrane. PGN is build of repeartoins of *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues. These repeations of glycan strands that are connected to each other by peptide stems that are attached to the Mur*N*Ac residue.

Bacteria live under large pressure of antibiotics and immune systems that are specialized in the recognition and killing of bacteria. Specific receptors have evolved in these immune systems that recognize specific conserved structures in bacteria. Therefore bacteria have also been evolved, which yielded structural alterations, that provide bacteria with immune evasion strategies. However, the human innate immune system is still able to recognize different structures in the bacterial cell wall. In this overview I will highlight in what level the human immune system is able to recognize parts of peptidoglycan.

Pattern recognition receptors are identified as proteins that recognize parts of the peptidoglycan. Peptidoglycan recognition proteins are the first receptors described in this article. The human innate immune system has 4 different types of PGRP. Next to these PGN specific receptors, we also have toll-like receptors. these TLRs are able to recognize specific microbial structures. They act on outer cell membranes and inner cell membranes and their signaling cascade after activation seems to be interconnected and very complex. Together with the TLR, Nod-like receptors (NLR) are the most well known pattern recognition receptors. Of the NLR family, Nod1 and Nod2, are known as being able to recognize DAP and MDP structures from the PGN layers in bacteria. There are results that also a relation between signaling pathways activated by Nod and TLR proteins. There are also controversial results that makes it very interesting to do further research into this subject, for example about the actual ligand that activates TLR2.

Abbreviations

PGN	-	Peptidoglycan
PRR	-	Pattern recognition receptor
PAMP	-	Pathogen associated molecular pattern
PBP	-	Penicillin binding protein
GT	-	Glycotransfer
TP	-	Transpeptidation
HMM	-	High molecular mass
LMM	-	Low molecular mass
IM	-	Inner membrane
OM	-	Outer membrane
LPS	-	Lipopolysschariden
OMP	-	Outer membrane proteins
Lpp	-	lipoprotein
TA	-	Teichoic acids
WTA	-	Wall teichoic acids
LTA	-	Lipoteichoic acids
PGRP	-	Peptidoglycan recognition protein
AMP	-	Antimicrobial peptide
TLR	-	Toll-like receptor
Nod	-	Nucleotide-binding oligomerization domain
MDP	-	Muramyl dipeptide
DAP	-	Diaminopimelic acid

Introduction

Bacteria are identified in many different shapes, like cocci and rods, and internal volumes from 10^{-2} to 10^{6} μm.[1] Compared to cells from any species, which do have an overall mutual similar shape and size, it sounds logical that the bacteria have different mechanisms for growth and maintaining cell shape and size. These mechanisms are till today interesting subjects for fundamental research in microbiology.

The major structural difference between bacteria and cells is found in the cell wall component peptidoglycan (PGN). Where cells are protected by a lipid bilayer, containing transmembrane proteins, bacteria are also protected by at least one lipid bilayer, but furthermore the cell wall contains a PGN layer that maintains cell shape and provides protection against pressure due to osmotic changes.[2] The PGN layer provides essential protection for bacteria, since inhibition of PGN synthesis by antibiotics has a bactericidal effect.[3] It seems obvious that peptidoglycan synthesis contains many alternative processes resulting in alterations in structure, because not all bacteria are sensitive to the same antibiotics that break down peptidoglycan or inhibit its synthesis.[4] Nowadays it is generally known that the synthesis of the peptidoglycan sacculus, surrounding the bacteria, is a dynamic process requiring synthases for the production of precursors and attachment to the peptidoglycan layer. Besides synthesis, also degradation is performed by enzymes to allow insertion of newly synthesized peptidoglycan for growth and divisions.[5] This dynamic process involves also spatial control of the activity of the enzymes responsible for synthesis and degradation.

Peptidoglycan comes in two forms in bacteria. First as a thin layer between two membranes in so called gram-negative bacteria. A second form of PGN is found as as a thick layer surrounding a membrane in so called gram-positive bacteria.[6] The thin peptidoglycan layer acts as a rigid structure that prevents burst of bacteria when the pressure from inside increases. The internal osmotic pressure can also be solved by the fluid between the two membranes in which the thin PGN layer is located. This so called periplasm space then acts as a active fluid that adapts to the osmotic environment by adjusting the water content and volume.[2]

Bacteria always had the tend to use host cells for reproduction. Over the years, mammals have evolved many ways to detect bacterial infection. This recognition is performed by parts of the immune system. The first detection takes place by the innate immune system. This part functions as a critical first line of defense, that is built around by pattern recognition receptors (PRR) that recognize pathogen associated molecular patterns (PAMP). PRRs from the innate immune system are germ-line encoded and thus detect only conserved parts of pathogens. Peptidoglycan is one of the most conserved structures in bacteria, that resulted in different receptors in mammals recognizing bacteria.[7]

The human innate immune system is evolved in such a matter that it can recognize many kinds of bacteria due to the conserved PGN structures. Special groups of receptors have been evolved to recognize parts of PGN from bacteria. However, lately more and more bacteria have been identified that are not (directly) recognized by the immune system. These bacterial species developed different processes in which the peptidoglycan layer is modificated in such a way that our ‘basic’ recognition receptors cannot identify PGN anymore. Current research is focusing on processes involved in the biosynthesis of PGN and how new unidentifiable residues are formed by these bacteria. Results of this research might improve treatment of bacterial infections that become more and more dangerous.

In this overview I will highlight in what level the human immune system is able to recognize parts of peptidoglycan. I will analyze where and how peptidoglycan is recognized in the human body and furthermore I will mention how bacteria became able to avoid recognition of their PGN by specialized receptors of the immune system.

Synthesis and Structure

Peptidoglycan biosynthesis is emerged in two cellular compartments. In the cytoplasm the formation, association and assembly of soluble precursors onto a lipid carrier occur. The created Lipid II is then translocated to the outer side of the cytoplasmic membrane by integral membrane proteins. Outside the membrane the last stages of biosynthesis are performed in which polymerization of Lipid II's glycan strands and cross-linking of its peptide stems are involved. These last stages are catalyzed by major peptidoglycan synthases called the penicillin binding proteins (PBPs). These PBPs are known as the targets of beta-lactam antibiotics.[8]

In the cytoplasm, UDP-Mur*N*Ac is generated from fructose-6-P by actions of the MurA and MurB enzymes that is followed by the binding of a stem peptide directly on this UDP-Mur*N*Ac molecule by ATP-dependent amino acid ligases MurC, D, E and F.[9] In the end, the product of the Mur ligases (UDP-Mur*N*Ac-pentapeptide) is the substrate for the integral mebraneprotein MraY, which generates the membrane bound Lipid I. The last step in this cytoplasmic Lipid II formation involves the transfer of a Glc*N*Ac molecule from UDP-Glc*N*Ac to Lipid I by MurG (Figure 1).[8]

Positioning of the MurG and F by interaction with actin homologue, MreB, reflects a cytoplasmic complex that explains the orientation of the Mur enzymes and restriction of diffusion of soluble peptidoglycan biosynthesis intermediates and also the transport of Lipid II to the periplasm where the last stages of peptidoglycan synthesis occur.[10]

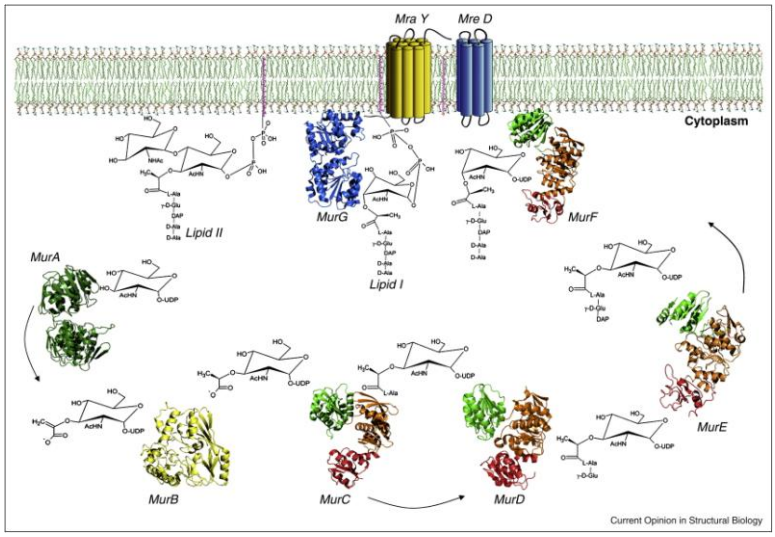

Figure 1. UDP-Glc*N*Ac is synthesized in the cytoplasm of bacteria. Seven Mur ligases are involved in this process and are located in such a way that no intermediates are diffused away.[8]

For finalizing the biosynthesis of peptidoglycan the lipid II is transferred to the outer side of the cytoplasmic membrane. For this transfer the the integral membrane protein FtsW, which is an protein involved in the bacterial division machinery, is a transporter of the lipid-linked peptidoglycan precursors across the cytoplasmic membrane.[11]

The last two conformations of peptidoglycan synthesis are polymerization of the glycan strands (glycotransfer, GT) and cross-linking of the stem peptides (transpeptidation, TP). These steps are mostly carried by high molecular mass (HMM) and low molecular mass (LMM) PBPs. It is difficult to call the specific functions for the PBPs in bacteria, since different bacteria species have different classified PBPs.[12]

HMM PBPs are built out of separate molecules and are mainly responsible for the peptidoglycan polymerization and insertion into the already existing cell wall. Depending on their structure and their catalytic activity they can be classified in either class A or B. Both classes have a transpeptidase activity, that catalyzes the cross-linking of two adjacent glycan strands. Class A is responsible for glycosyltransferase, class B is believed to interact with other proteins involved in the cell-cycle to influence the morphogenesis.

LMM PBPs are classified in class C with numbered subdivisions (C1, C2, C..). All LMM PBPs posses different functions, from transpeptidase and transglycosylase to functions in cell elongation and division and recycling.[12]

A DD-peptidase activity in PBPs is common, this can be a DD-transpeptidase, DD-carboxypeptidase or a DD-endopeptidase activity. The carboxypeptidase and transpeptidase reaction consists of three parts. First a noncovalent binding between the enzyme and the peptidoglycan pentapeptide stem is set up, called a Henri-

Michaelis complex. Then acylation takes place so a C-terminal D-ala is released from the D-ala-D-ala peptide tail, that is explained later. Finally, this tail hydrolises with the release of a shortened peptide (carboxypeptidation). The other possibility is cross-linking with a second peptidoglycan peptide stem (transpeptidation).

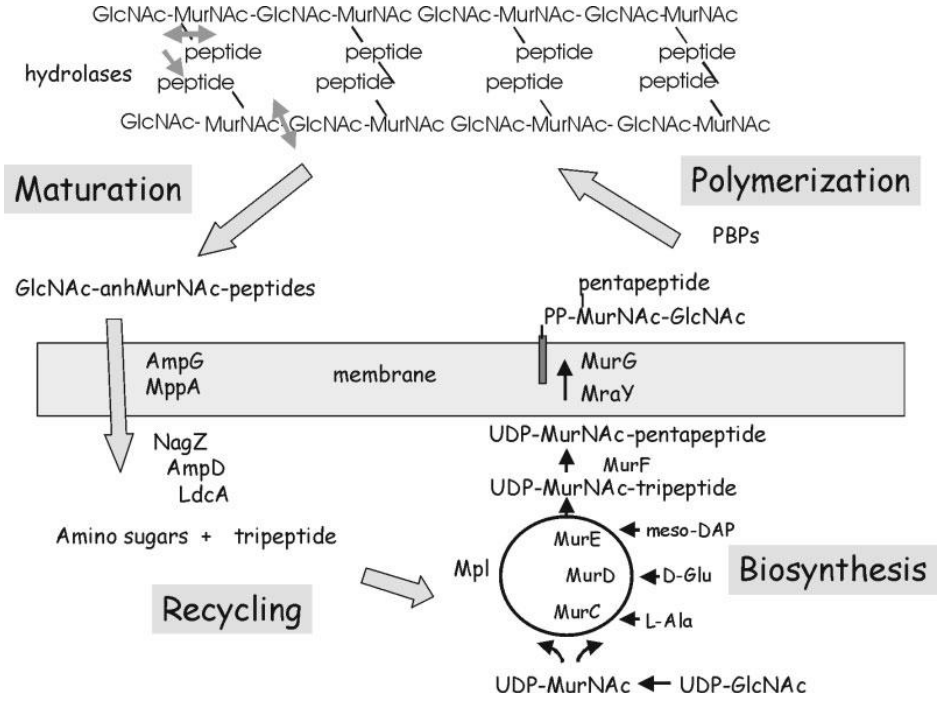

Figure 2. Peptidoglycan metabolism in *E. coli*. Peptidoglycan is synthesized in the cytoplasm, which is followed by translocation to the outer side of the membrane. From then PBPs are responsible for the polymerization reactions in the PGN layer. In periods of growth and division, PGN is cleaved and recycled .[9]

During divisions and growth of bacteria peptidoglycan degradation and thus restructuring is necessary. Bacteria are able to do this efficient, by balanced activities of murein-degrading and murein synthesizing processes. Soluble SltY enzymes are able to cleave between the Mur*N*Ac and Glc*N*Ac residues, as do lysozymes and muramidases too. SltY induces remodelling of the murein residues that resulted in the turnover process. Peptides are released in the extracellular environment and the turnover products, Glc*N*Ac-anhMur*N*Ac-tetra and –tripeptides are imported into the cytoplasm and efficiently re-used in a process that is known as the recycling pathway for peptidoglycan. A large set of enzymes is involved in this process that results reproduction of Glc*N*Ac, Mur*N*Ac and D-ala and murein tripeptides. These products then re-enter the current peptidoglycan synthesis process (Figure 2).[9]

There are two groups in which the cell wall architecture is divided. Cell walls belonging to the gram-negative and the gram-positive bacteria. These groups originate from the period when Christian Gram (1884) developed a staining procedure in which bacteria could be divided. The Gram-positive bacteria retain the stain after washing and the gram-negative bacteria do not. The fundamental cause of this difference lies in structural differences in the cell wall of the bacteria.[6]

Gram-negative bacteria are surrounded by an inner cell membrane (IM) and an outer cell membrane (OM). Between these two membranes a thin layer of peptidoglycan is positioned. The outer membrane (OM) is composed of glycolipids, mainly lipopolysacchariden (LPS) instead of normal phospholipids.[13] It contains a lot of different outer membrane proteins (OMPs) like lipoproteins and β-barrel pores.[14] The most obvious function of the OM is to serve as a protective barrier and LPS play a critical role in this barrier function. LPS is a glucosamine disaccharide with six or seven acyl chains. This is connected to a polysaccharide core with at the end a domain called the O-antigen.[15]

Between the OM and the IM a thin peptidoglycan layer is positioned. This rigid layer determines the cell shape. Without the peptidoglycan layer, due to breakdown by antibiotics for example, the bacterium lyses because of the huge pressure from the cytoplasm and osmotic pressure. The outer membrane is anchored to the rigid peptidoglycan layer with major murein lipoproteins (Lpp).[16, 17]

The inner cell membrane (IM) of bacteria is a phospholipid bilayer that contains all the proteins that function in energy production, lipid formation, protein secretion and transport.[6]
All proteins for the cell envelope are synthesized in the cytoplasm or at the inner surface of the IM. Subsequently, the proteins are transported towards the periplasm and peptidoglycan layer or the OM, like it is the case for the peptidoglycan building units.

Gram-positive bacteria lack an outer cell membrane. Instead of this outer membrane, they are surrounded by thick layers of peptidoglycan. Through these thick peptidoglycan layers, long anionic polymers are threaded. These polymers are called teichoic acids (TA) and consist largely of glycerol phosphate.[6]
Gram positive bacteria also contain different anchored proteins, many of which are directly involved in adhesion an invasion.[18]
The structure of peptidoglycan itself in gram-positive bacteria is the same as in gram-negative bacteria, as in a disaccharide-peptide repeat, which are coupled by glycosidic bonds to form linear strands that are cross-linked through a binding of the peptide stem to the disaccharide repeat of another strand.[6] The major difference between gram-positive and gram-negative peptidoglycan is the thickness of the PGN layers. Gram-positive peptidoglycan is 30-100 nm thick, where gram-negative peptidoglycan is 2-7 nm thick.
Among almost all bacteria species variation in the detailed peptidoglycan structure is detected. Also variation in cross-linking between peptide stems and glycan strands is found.[19]

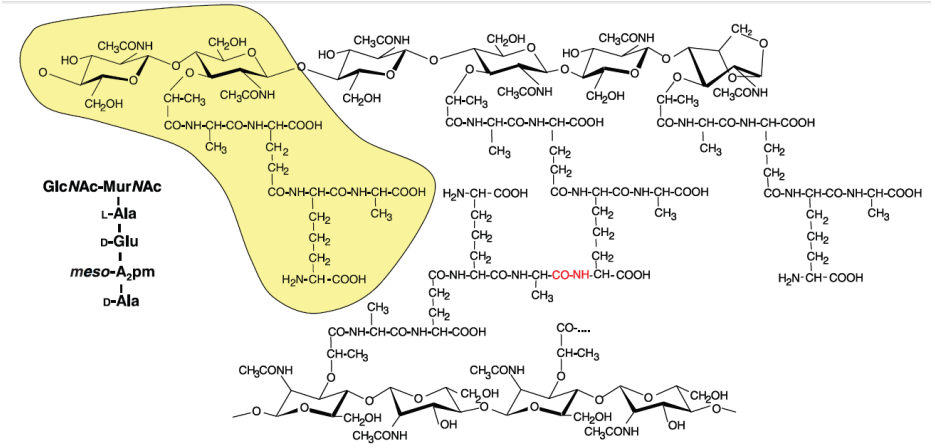

Figure 3. Structure of peptidoglycan in *E. coli*. The glycan strands consist of *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues. Inbetween, peptide stems are cross-linked to each other. There is variation in cross-linking between the peptide stems[21].

The glycan strands of peptidoglycan are usually built up by repeations of *N*-acetylglucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) residues. The D-lactoyl group of each Mur*N*Ac residue is substituded by a peptide stem with a L-Ala-y-D-Glu-*meso*-A_2pm-D-Ala-D-Ala composition. This composition alters between different species of bacteria. The cross-linking between glycan strands normally occurs between the fourth peptide D-ala, and the third diamino acid of the other strand, through direct binding or a short peptide bridge (Figure 3).[19] Glycan strands are in general between 10 to 250 disaccharide units long, but the length of the glycan strands does not indicate the thickness of the peptidoglycan layers, since there are short glycan strands in thick layers (*S. aureus)* but also long strands (*B. subtilis)* in gram-negative thin peptidoglycan layers.[19]

Structural variations

Peptide stem variations

The peptide stem is a structure that is sensitive for a variation. Two causes are known for this variation. First variation due to the actions of Mur ligases, the enzymes that are responsible for the biosynthesis, cause variation in the peptide stem. But also steps later in biosynthesis of peptidoglycan cause variation in the peptide stem.
For example, the MurC ligase is responsible for the addition of the first peptide, that is in almost every case L-ala. But is some rare situations Gly or L-Ser is bound on the first peptide position. The most variation in the peptide stem is found at the third position. MurE is responsible for positioning the third amino acid, that in general is *meso*-A_2pm for most gram-negative bacteria, or L-Lys for most gram-positive bacteria. There are species known where other diamino acids, like L-Orn and *meso*-lanthionine, but also monoamino acids are positioned as third peptide.[19] The specificity of MurE differs in other species, where in *S. aureus* and *E. coli* only

respectively L-Lys and *meso*-A_2pm are added, is MurE in other species less specific and capable of inserting different amino acids into the peptide stem.

At position 4 and 5 in almost every case a dipeptide is added. This dipeptide, D-Ala-D-Ala, is generated by an enzyme called Ddl enzyme and the addition to the peptide stem is carried out by MurF ligase.[20] There are species where D-Lac or D-Ser is found on position 5 that are resisitant to vancomycin due to a lower affinity.[21]

Other variation in the peptidoglycan structure is caused after the Mur ligases, so this is in the Lipid II level. This variation expresses itselves at position 2 and 3, and most enzymes that carry out this variation are unknown.[19] It has been shown that lipid II is a substrate for amidation in *Mycobacterium Smegmatis*.[22] But also sometimes parts of the peptides in the peptide stem are hydroxylated, that depends on the oxygen supply during the growth of the bacterium.[23] In other species parts of the peptide stem are acetylated.[24]

Cross-link variations

As mentioned before there is a large degree of possible alterations in the composition of the peptide stem on the glycan strands. But also the cross-linking, with which the glycan strands are connected, carry out some variations, especially in gram positive bacteria. Also the interpeptide bridge that sometimes occurs shows variations. Cross-linking between peptide stems are divided in two main groups. The first group shows cross-linking between the amino group at position 3 on the peptide side-chain with the residue on position 4, mostly D-Ala, of another peptide side-chain (3-4 cross-linkage). This can be either a direct binding, as in most gram-negative bacteria, but also through a peptide bridge, mostly in gram-positive bacteria.

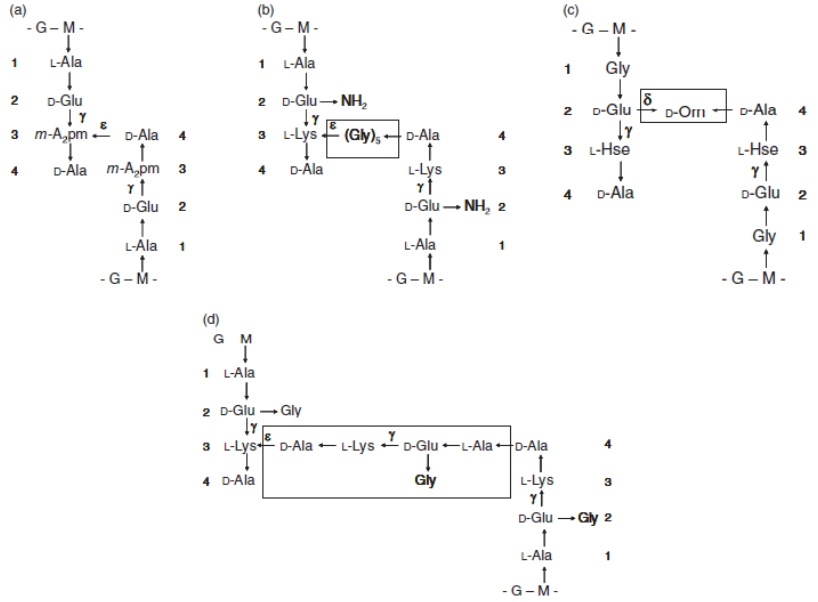

Figure 4. Examples of cross-linkage and interpeptide bridge. (a) *E. coli* (direct 3-4 cross-link); (b) *S. Aureus* (3-4 cross-link with a interpeptide bridge); (c) *C. pointsettiae* (2-4 cross-link with interpeptide bridge); (d) *M. luteus* (3-4 cross-link with a bridge consisting of a peptide stem).[19]

The second group, a smaller group, shows cross-linking between the amino residue at postion 2 and position 4 of the other peptide side chain (2-4 cross-linkage). This group is limited to corynebacteria and an interpeptide bridge is needed. This cross-linking reactions are catalyzed by penicillin binding proteins (PBPs). The length of interpeptide bridges can vary from 1 to 7 amino acid residues, and several amino acids are involved.[12, 19] The variation in cross linkage can be very different. For example in *M. luteus* is the peptide stem detached from the Mur*N*Ac residue and forms a peptide bridge (Figure 4).[25]

Backbone variations

As mentioned before, the glycan strands of peptidoglycan are generated by polymerization of lipid II. That results in glycan strands consisting of alternating Glc*N*Ac and Mur*N*Ac residues. However, so far it is never seen that glycan strands remain unmodified. This is because glycan strands always get modified after their synthesis or bind to other cell-wall polymers. *N*-deacetylation, *O*-acetylation, *N*-glycolylation are the most well known modifications of the glycan strands and the enzymes responsible for these modifications are also identified.[26]

In several bacterial species non-actetylated glucosamine and muramic acid residues are identified.[27] These nonacetylated residues are correlated with lysozyme resistance.[28] Deacetylation takes place after polymerization of the residues, since there has no deacetylated precursors been identified and deacetylases localize outside the

cytoplasmic membrane.[29] *N*-glycosylation has also been described, and is also used in bacterial taxonomy for certain species. The most common kinds of bacteria that show glycosylation in their peptidoglycan are the *Mycobacterium, Rhodococcus, Tsukamurella, Gordonia, Nocardia, Skermania,* and *Dietzia*.[30] These bacteria contain mycolic acids and thus are called the mycolata. Unlike *N*-deacteylation, *N*-glycosylation as modification is introduced at the last cytoplasmic step of peptidoglycan synthesis (UDP-Mur*N*Ac-pentapeptide) and is catalyzed by mono-oxygenase (hydroxylase) in the presence of molecular oxygen and NADPH.[31] The biological role of glycosylated residues in the backbone remains unclear so far, although it has been speculated that the extra hydroxylgroup on glycolate has something to do with bonding within the cell envelope and thus influences stability of the envelope.[32] A *M. smegmatis* that was mutated and lacked the *N*-glycosylation seemed to be more sensitive to lysozyme and therefore *N*-glycosylation might have the same funtion as *N*-deacetylation in other species.
The third widely studied backbone modification is the *O*-acetylation. Species with this modification have a peptidoglycan layer that contains an extra acetyl group linked to a muramic acid residue. *O*-acetylation of Glc*N*Ac residues have not been identified so far. This modification occurs in both gram-positive and gram-negative bacteria, like *S. aureus* and *N. gonorrhoeae*. The source of this extra acetyl group is still being discussed.[26] In *S. aureus* and *S. pneumoniae* the *O*- acetylation of the peptidoglycan contributed to lysozyme resistance, and mutant strains lacking this modification got more sensitive to lysozymes.[33] Bera et al (2007) showed that besides *O*-acetylation and *N*-deacetylation, also the teichoic acid degree in the cell wall contributed to lysozyme resistance.[34]

Obviously it is very hard for bacteriologists to keep the differences in all the species clear, so from the beginning there are some taxonomic implications introduced.[23] To classify the variations in peptidoglycan structure there is a three-digit system developed. The three-digit system starts with an A or an B, that stands for 3-4 cross-linkage and 2-4 cross-linkage respectively. The second digit is a number that gives information about the type of inter peptide bridge or the lack of it. The third and last digit is a Greek letter that indicates the amino acid found at the third position, probably because here the largest variation exists.[23]

Peptide stem and glyccan strands functioning as point of anchoring
Bacteria have proteins on their surface to interact with the environment. These proteins need mainstay in the cell wall. In gram-positive bacteria, cell wall polymers are anchored into the peptidogycan. Liu and Gotschlich discovered in 1967 an muramic acid-6-phosopate, that evidently was part of a bond between the polymers and glycan strands. Most well known polymers are the teichoic acids, teichuronic acids as well as capsular polysaccharides and adhesions.[6, 26] The display of bacterial surface proteins involves covalent or noncovalent interactions with either the peptidoglycan or the secondary wall polymers, but most surface proteins in gram positive bacteria are attached covalently to the stem peptides. Teichoic acids play also an important role in the recognition of bacteria by the human immune system. Two types of teichoic acids are identified. The wall teichoic acids (WTA) that are attached to peptidoglycan and lipoteichoic acids (LTA) that are bound in the cell membrane and reach through the peptidoglycan.[6]
In gram-negative bacteria the surface proteins are localized in the outer membrane, which on their turn are connected to the inner peptidoglycan layer.[35] Only lipoprotein (Lpp), murein lipoprotein and Braun's lipoprotein are known as covalently bound to the peptidoglycan in gram negative bacteria, of which Lpp is the most abundant [6]. They all fulfill the same function: attachment of the OM to the rigid peptidoglycan layer.

The information above indicates that the peptidoglycan layer in the cell wall of bacteria is of great importance for the existence of bacteria. The synthesis is tightly regulated by Mur enzymes as builders of the precursors, and PBPs as building the precursors into the peptidoglycan layer. As it became clear in the last part, there is great variation in the detailed structure of the peptidoglycan layer. This variation is the result of differences in enzyme specificity between different species, but also the state in which bacteria live influences the level of modifications in peptidoglycan structure. Alterations in structure are found in the backbone of glycan strands, consisting of the repeating Mur*N*Ac and Glc*N*Ac residues, in the cross-linked peptide stems between the glycan strands and also in the type of cross-linkage.
Another function that is not discussed in detail yet is immune evasion. The human immune system is one of the most complicated immune systems and for some bacteria to survive, they need to avoid getting killed by this

immune system. As result of this pressure bacteria evolve fast and this explains the large amount of variations between species of bacteria. The human immune system is able to degrade bacteria in small parts and expose these parts to pattern recognition molecules. Also peptidoglycan is degraded by enzymes from the immune system and specific patterns of peptidoglycan are recognized.
In the following part the way the immune system acts against bacteria will be discussed. Especially the recognition of peptidoglycan by pattern recognition receptors will be described.

Peptidoglycan recognition

The human immune system is divided in an innate and an adaptive part. The fundamental part of the adaptive immunity consists of cells with highly specific receptors that can recognize any antigenic molecule. This immunity is extended with specific T cells and antibodies that are products of B cells that are activated by those specific receptors. The weakest point of the adaptive immunity is the time between recognition and the effective response by T cells and antibodies. At that point the innate immunity takes part. The innate immune system has several mechanisms to prevent infection. The innate immunity has been highly conserved, but also has developed throughout evolution.

Receptors are developed to be a sensor for pathogenic and non-pathogenic microbes. They recognize fundamental parts of microbes and are hardly susceptible to modification. These so-called pattern recognition receptors (PRR) recognize the so-called pathogen-associated molecular patterns (PAMPs). These motifs are widely conserved across many different microbes. Peptidoglycan, the fundamental component of the cell wall in gram-positive and gram-negative bacteria is a typical example of a molecular pattern for our immune system to recognize. Its unique structural components are Glc*N*Ac and Mur*N*Ac with the peptide stems that are responsible for the cross linking that are sources of variation. As mentioned before, this variation can be caused either by modification and composition. Gram-positive bacteria for example contain a lysine residue at the second position in the peptide stem and gram-negative have a diaminopelic (DAP) acid residue at that position. PRRs are able to recognize these structures. PRRs are also found in other eukaryotes like mice and flies.[7]

PGRP

PPRs that recognize peptidoglycan are called peptidoglycan recognition proteins (PGRP). In 1996, PGRP is first discovered. It was found to bind peptidoglycan when it was purified from the silkworm *Bombyx mori*.[36] For investigation of the innate immune system insects are used, since they only carry innate mechanisms and vertebrates' immune responses are 'soiled' with adaptive mechanisms.

PGRPs in insects can bind to peptidoglycan of gram-positive bacteria, that activates the prophenoloxidase pathway. The prophenoloxidase pathway is one of the antimicrobial mechanisms of insects. When the PGRPs from mice, flies and humans were cloned and sequenced it was shown that all PGRPs were able to bind gram-positive bacteria and also similarities in sequences were found. This indicated that this protein is obiously conserved from insects to mammals and it also indicates that the human innate immunity partly origins from insects.[37]

In insects, anti-microbial peptides (AMPs) are an important part of the immune system.[38] The innate immunity in *Drosophila* showed similarities with animal innate immune responses, and together with the complete sequenced genome of *Drosophila*, these insects got more and more important in immune investigation.[39] Clones and sequencing of PGRP genes from the *Drosophila melanogaster* led to the identification of different PGRP gene families in insects, but also in mammals and fish.[40] *D. melanogaster* is known to have 13 PGRP genes that are coding for 19 proteins. In mammals on the other hand 4 PGRP genes are known, PGLYRP1, PGLYRP2, PGLYRP3 and PGLYRP4.[41] All peptidoglycan receptors do have a binding domain that specifically binds peptidoglycan, and some receptors have two binding domains. There are also bigger PGRPs with an additional amino sequence. Soluble PGRPs are known to act as linkers of PGN recognition and intracellular signalling. One of the two binding sites is highly conserved to recognize PGN, and a binding site that has a variable location behind the PGN binding site that can bind to effector proteins inside cells.[42]

In flies, binding of peptidoglycan results in production of AMPs. PGRPs in the *Drosophila* can discriminate different compositions of peptidoglycan. Bacteria that have a lysine residue at the second position in the peptidestem, gram-positive, can be discriminated from gram-negative bacteria, where a *meso*-diaminopimelic acid posseses this position. When a lysine residue is found at this place, the so-called Toll pathway is activated that regulates AMP production. In case of recognition of a *meso*-diaminopimelic acid another pathway, called IMD pathway, is activated.

PGLYRP1 is the shortest transcript of the four PGRPs in mammals. PGLYRP1 is mainly expressed in the bone marrow and polymorphic leukocytes and a little in intestinal M-cells and non-immunologic cells like epithelial cells and fibroblasts.[43]

PGLYRP2 was called PGRP-L before because of it is the longest transcript of the four. It is expressed in the liver where it is secreted into the blood, but also in lower oral and intestinal epithelial cells. The PGLYRP2 gene codes for one protein that has two functions, namely the PGLYRP2 protein and the *N*-acetylmuramoyl-L-alanine amidase in serum. PGLYRP2 expression depends on activation of the nucleotide-binding oligomerization domain (Nod)2, which will be discussed later.[43] The amidase activity of PGLYRP2 contains the hydrolyzation of the lactyl bond between Mur*N*Ac and the L-alanine in peptidoglycan. The substrates for this enzyme are muramyl-tripeptides. Fewer peptides are not hydrolyzed. The amidase activity is zinc dependent cause PGLYRP2 has a cysteine in the catalytic site and other PGRPs have a serine residue on that position.[44]
PGLYRP3 and PGLYRP4 are selectively expressed in many different tissues, from skin epidermis and hair follicles to different glands in the dermis or in the stomach and esophagus. Activation of TLR2, TLR4, Nod1 and Nod2 increases the expression of PGLYRP3 and 4 in keratinocytes, fibroblasts and oral epithelial cells.[43]
Human PGLYRP1, 3 and 4 all have bacteriocidal and bacteriostatic activity for different gram-positive and -negative bacteria. Heterodimerization of PGLYRP3 and 4 also forms a complex with this bacteriocidal activity. The human PGRPs form a separate class of bacteriocidal proteins, since they are 3-8 times bigger than other anti microbial peptides (AMPs). Additionally, they also need a *N*-glycosylation for the bacteriocidal activity and they also differ in mechanism: PGRPs binds to peptidoglycan, probably to inhibit peptidoglycan synthesis, and AMPs permeabilize the membrane of bacteria. PGRPs are often present in similar parts in the body as AMPs to kill bacteria synergistically.[45]
The binding groove of these four receptors recognizes the muramyl pentapeptide or tetrapeptide, that is part of the peptidoglycan building blocks. Te binding groove is not able to bind muramyl-dipeptide or a peptide without the Mur*N*Ac residue.[46] Binding results in a conformational change in the binding groove that locks the peptide stem into the receptor. PGRPs are able to identify different amino acids in the peptide stem, but they also can bind to other proteins anchored in the peptidoglycan, such as LPS and lipoteichoic acids (LTA). These molecules usually bind to other parts than the PG binding groove of the PGRPs.[47] Binding outside the peptide groove of PGRPs normally initiates oligomerization or dimers of PGRPs. Besides PGRPs, also other receptors have been identified that are able to recognize peptidoglycan.

TLR

The biggest part of the innate immunity surveillance in the blood is done by circulating monocytes that recognize specific PAMPs. Toll-like receptors play an important role in this microbe pattern recognition and binding. TLRs are found on the cell surface and in endosomal compartments, sensing specific microbial and viral structures. The most important intracellular protein that starts the signaling cascade for TLR is MyD88. MyD88 is connecting all TLRs to its associated transduction pathway, except TLR3. TLR4 has an extra adaptor protein with a Toll/IL-1R domain that induces IFN-β. Next to the production of anti-inflammatory proteins, activation of the signaling pathways results in changes in surface protein production and expression.[48]

The discovery of the Toll-like receptors increased our understanding of how our innate immune system senses and reacts to microbes. TLRs are also pattern recognition receptors that can be activated by pathogen associated molecular patterns, which activates transcription factors as NF-κB. These transcription factors in their turn induce pro-inflammatory cytokine and cell-surface protein production.[49]
TLR2 and TLR 4 are known to be the most important in bacterial cell wall recognition. TLR4 binds LPS that is anchored in the cell wall of gram-negative bacteria. The role of TLR2 in peptidoglycan recognition is controversial. The complex structure of peptidoglycan and the modifications in the backbone or the peptide stem together with anchored cell wall proteins makes it difficult to determine the true ligand for TLR2 activation.
Each TLR is a type 1 membrane protein with an extracellular leucine-rich domain that functions as the binding domain for the ligand. The intracellular tail of TLRs contain a highly conserved Toll/IL-1R (TIR) homology domain that in case of activation recruits MyD88. MyD88 binds to the tail and then activates an intracellular pathway.[50] The homologous cytoplasmic domains are not functional equivalent to each other with respect to signalling, but it is suggested that different TLR pairs activate different pathways.[50]
TLR2 probably is evolved in such a way that it only recognizes a limited amount of lysine-containing PGN residues from gram-positive bacteria to avoid over activation of innate immune responses.[51] On the other hand, a wide range of gram-negative DAP-containing peptidoglycan is recognized by the TLR2. This is probably because gram-negative bacteria contain a thin layer of petpidoglycan, so over activation of innate immune

responses is less likely.[51] TLR2 is able to discriminate gram-positive and gram-negative bacteria and it appeared to be an unique mechanism. Where one TLR2 receptor recognizes lysine- as well as DAP-containing PGN but responds differently to these molecules due to different binding ways.[51] NOD receptors have evolved in two separate receptors to discriminate between gram-positive and –negative and also the PGRPs discriminate, but with two strategies. One on lysine or DAP content, and a second on the peptide cross-bridge.
TLR2 seems to react on wall proteins in a way similar to LPS. TLR4 reacts to LPS together with CD14. But also PGN and lipoteichoic acids activate macrophages through CD14 co-activation. Even when the heat inactivated gram-positive *S. aureus* is exposed to TLR2 and TLR4 containing cells, TLR2 and CD14 but not TLR4 is activated.[52]
Another study showed that TLR2 interacts with other TLRs, like TLR1 and TLR6, to mediate different responses. When cells with mutated TLR2 and TLR6 were exposed to peptidoglycan, the original peptidoglycan-dependent TNF-α production was inhibited. But when cells are stimulated with Pam_3CSK_4, a lipoprotein that acts as lipoprotein found in gram-positive and gram-negative cell walls, only a TLR2 dependent TNF-α production is measured.[50] TLR6 had also no effect on LPS-dependent TNF-α production, which is driven by TLR4.
Next to a cytokine response, also the expression level of TLR on monocytes changes after TLR activation. Exposure of monocytes to gram-positive bacteria, *S. aureus* and *S. pneumonia*, enhanced TLR2 but not TLR4 expression on the cell surface. Remarkably fact is that gram-negative bacteria in low concentrations also enhanced TLR2 expression, but in higher concentrations there was no increased TLR2 expression measured.[53] TLR4 is down-regulated when monocytes are exposed to gram-negative bacteria, probably because of a induction of LPS tolerance.[54]
Other studies argue that TLR is not activated by the pure PGN itself but by PGN contaminated with other surface proteins, like lipoteichoic acids. When PGN was purified, TLR2 dependent NF-κB activation decreased or was lost.[49] This study believes that TLR2 activation by gram-negative bacteria is mostly due to covalently bound lipoproteins in the peptidoglycan layer. TLR2 activation by gram-positive bacteria should be due to lipoteichoic acids from the thick peptidoglycan layer. Another study tried to disprove these findings, by analyse their PGN fraction by mass-spectrometry and show the absence of LTA and other contaminants. TLR2 transfected HEK293 cells showed TLR2-dependent NF-κB activation by purified PGN.[55] Long et al. investigated the intracellular activation of pathways and cytokine production after exposure to different TLR2 ligands. They found that pure LTA caused a delayed activation of the MAPK NF-κB pathway. Also LTA was not able to induce the robust leukocyte recruitment, as other ligands did. This suggested that there is a difference in cellular response between TLR2 ligands.[56]
This wide range of molecules that possibly interact with TLR2, and the existence of twelve other TLRs suggests that these receptors have different activation strategies that are interacting with each other.[50]
It was found that modification of the carboxylic acids iso-Glu and DAP had large influences on the binding. Probably modification is used by bacteria to avoid recognition by TLR2, but other receptors, like NOD, react different on these modifications. In 2004 researchers also found that there is a certain crosstalk between the TLR2 and Nod receptors.

Nod

Toll-like receptors are pattern recognition receptors that act on the cell surface membrane and inner cell membranes of different cells, including monocytes in the blood. But our immune system is also sensing molecules that already passed the outer cell membrane and are in the cytoplasm. Therefore we have intracellular Toll like receptors. But also receptors from the Nod-like receptor (NLR) family. Two well-studied NLR proteins are the bacterial peptidoglycan receptors Nod1 and Nod2.[57] Nod receptors are cytoplasmic proteins that have a nucleotide-binding oligomerization domain (Nod). Specifically, Nod receptors play important roles in signalling pathways that are triggered by sensing bacterial cell wall compounds, like PGN, and result in pro- and anti-inflammatory cytokine productions. They participate in resistance to bacterial species, antigen presentation and induction of specific T cell responses and thus are primarily expressed in immune cells.[58]

NLRs are a large family of PRRs with a similar architecture. At the N-terminus there is a binding domain that consists of protein-protein interaction domains, that for example recruit caspases (CARD). Then they have a intermediate NOD domain that is able to bind nucleotides and mediates self-oligomerization. C-terminal leucine rich repletion motifs recognize the conserved PAMPs.[59] Based on differences in genetics NLRs are classified into three families, CARD-containing NODS, PYD-containing NALPS or BIR containing NAIPS. These families differ in the effector domain. These differences allow the NOD receptors to activate a wide array of cellular signalling pathways. CARD and PYR containing Nods posses homophilic association in contrast to BIR-containing NAIPS that are not involved in this homophilic association and the associated domains in CIITA domain contains a transcriptional activation domain for nuclear factors that are required for MHC-II gene regulation.[58]

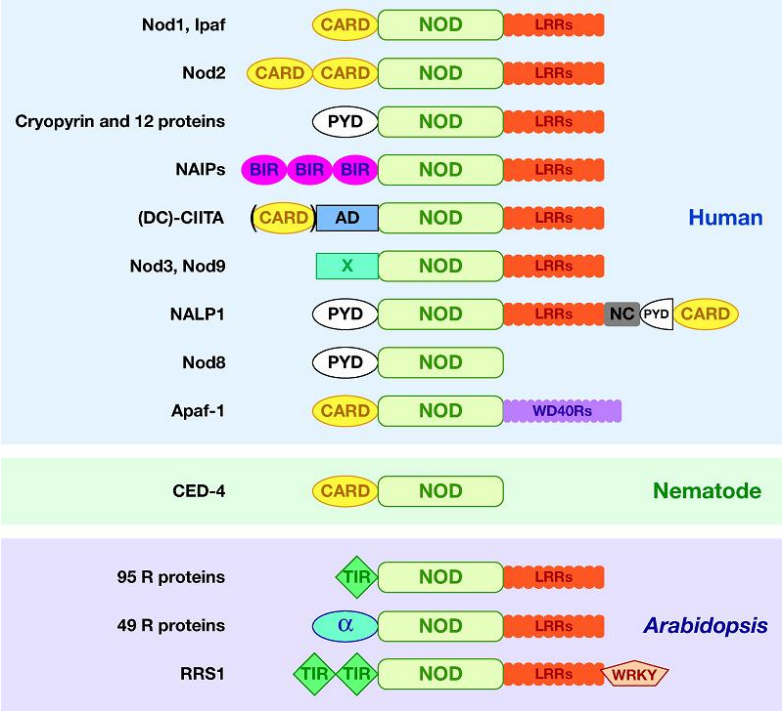

Figure 5. Domain structure of Nods. Nods consist of a centrally located nucleotide-binding oligomerization domain (Nod) and a C-terminal located leucine rich domain that recognizes ligands. At the N-terminal, Nods have a lot of variation in domains associated .[58]

At the C-terminus of Nod, the leucine rich residue (LRR) is located (Figure 5). The LRR are responsible for the recognition of the passing PAMPs. Within these LRRs, variable residues are found that are essential for recognition of specific PAMPs, as MDP. There are mutations that are pointed out to reduce the ability of recognizing MDP. The areas in which these mutation are found are suggested to be interacting with MDP directly, but there are also theories about indirect binding of MDP by cofactors. Some of the mutations in the LRR region are only found in Crohn's disease (CD) patients, implying Nod receptors as risk factor for CD.[60]
The LRR at the C-terminus are known to fold back on the intermediate Nod-domains to prevent spontaneous activation of this domain. When the LRRs recognize PAMPs, the receptor undergoes a conformational change in which oligomerization is triggered. Also the effector domain is exposed, resulting in recruitment of PYD or CARD-containing effector molecules.[61]

Ligand recognition by Nod1 or Nod2 induces recruitment of RIP2 that interacts with the CARD domain. The CARD-containing serine-threonine kinase is responsible for activation of the IKK complex, that in turn degradates the NF-κB inhibitor IKBα, that results in translocation of active NF-κB to the nucleus that activates specific genes in there.[57] RIP2 seems to be absolutely required for the induction of inflammatory responses after activation, but another process that is dependent on Nod signalling, autophagy, seems not to require RIP2.[57] Next to the NF-κB pathway activated by Nod receptors, also MAP kinase, procaspase-9, ERK and JNK pathways are stimulated as discussed later.[61, 62] Some studies also suggest a role for RIP2 in TLR signalling, when TLR signalling was reduced in RIP2 deficient macrophages.[63] Other NLRs are responsible for the activation of diverse signalling pathways, like CIITA transcription factor activation activates MHCII genes and Nalp1 family of NLRs activates the caspase-1.[59]

Several studies proved that there is a certain synergistic effect of LPS recognition together with the recognition of PGN by Nod receptors.[64] Probably TNF-α is transcribed when NOD is activated by muramyl acids and NF-κB translocates to the nucleus, but the translation and secretion of TNF-α is induced after co-incubation of a TLR ligand, like LPS.[65]
The NLR-family has two major receptors that take part in bacterial sensing inside the cell. Nod1 is a meso-diaminopimelic acid (DAP) containing PGN recognition receptor. DAP-containing PGN is produced by gram-negative and specific gram-positive bacteria, including *Listeria* and *Bacillus* families. The second muramyl dipeptide (MDP) and lysine containing PGN recognizing Nod2 senses almost every species of bacteria (Figure 6).

As noticed before, peptidoglycan structure consist of parallel glycan strands composed of alternating amino sugars, Glc*N*Ac and Mur*N*Ac, that are cross-linked by peptide stems that are connected to the Mur*N*Ac residue. Previous experiments showed that large purified PGN structures are poor ligands for Nod receptors. Generally, smaller peptide fragments posses better stimulatory activity than large PGN molecules.[58]
In bacteria, the peptidoglycan structure is often remodelled because of divisions and growth, and during these phases bacterial enzymes produce intermediate PGN molecules that contain the DAP and MDP structure. But also lysozymes in host body fluids as tears and blood and enzymes from phagocytes are involved in the degradation of bacterial PGN by cleaving the glycan strands, producing smaller PGN molecules, containing DAP, but also MDP structures recognized by Nod2.[66] The DAP core structure in PGN seems to be highly resistant against heat, acidic and organic extraction treatments. That can explain why a lot of commercial preparations of PGN and other bacterial components contain still Nod1 stimulatory molecules.
Enzymes in the host are N-acetylmuramoyl-L-alanine amidases that cleave the bond between the peptide stem and Mur*N*Ac residue. Other enzymes, AmiA and AmpD are involved in recycling bacterial PGN. It is suggested that this process degradates the MDP structures in peptidoglycan, but DAP structures are maintained.[67] So far, only bacterial enzymes are known, like DL-carboxypeptidases, which are able to cleave both DAP and MDP structures. All PGN fragments detected in host fluids are derived complete PGN and amidase-cleaved structures.[58] The fact that only bacteria have those enzymes that can cleave Nod1 and Nod2 stimulatory molecules, shows that degradation of endogenous cell wall structures can be a strategy to evade Nod1 and Nod2-mediated host defence.[58]

Activation initiates a conformational change of the receptors that allows oligomerization and exposure of the CARD-domain that interacts RIP2(RICK). RIP2 is known to be involved in Nod1 and Nod2 NF-κB activation. Specifically Nod1 activation induces an interaction between Nod1, RIP2 and IKK, of which it is suggested to induce down-regulating intracellular responses. Also it is reported that Nod1 activation by bacteria induces the c-Jun N-terminal kinases (JNKs).[68]
Caspase-9 is showed to be inducing apoptosis after binding of pro-caspase 9 to the CARD domain of the Nod1 receptor but further research is needed for the role of NOD receptors in apoptosis. Agonists of Nod1, but also Nod2 show a stimulation of IL-1β secretion by human monocytes. Pro-IL-1β is cleaved by capsase-1, which takes place in so-called inflammasomes. Nod1 enhances this processing of pro-IL-1β by heterophilic CARD-CARD interactions between Nod1, RIP2 and pro-caspase-1.[69]
Nod2 is also known to induce caspase 9 dependent apoptosis, but not by direct interaction by caspase 9.[69] Caspase 9 activity did not interfere with the NF-κB activation pathway.[61]
The intracellular pathways involved after Nod activation seem to be highly controlled as many studies are performed to identify intermediates in the activation process of transcription factors.
NF-κB is activated after Nod activation. But for Nod1 other intermediate molecules are involved, compared to Nod2. TRIP-6 interacts with RIP2 on the Nod1 receptor and Nod2 activates extracellular signal-regulated kinase proteins (ERKs) upon MDP triggering of the Nod2 receptor, that in turn results in phosphorylation of the transcription factor Elk-1.[70]
GRIM-19 is required for NF-κB activation by Nod2 recognizing MDP. Also TAK1 is an essential component of signalling pathways downstream the Nod2 activation that activate inflammatory cytokines.[71]

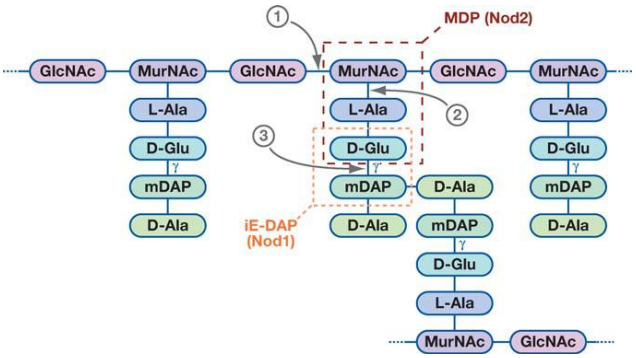

Figure 6. Generation of MDP and iE-DAP structures from peptidoglycan. These two are the main ligands for Nod activation. The numbers in the circles show the cleavage sites for muramidases. 1.muramidases 2. N-acetylmuramoyl-L-alanine amidases 3. endopeptidases.[58]

Other antibody neutralizing studies showed that this wide array of intracellular activity is in the end responsible for the key pro-inflammatory cytokines, TNF-α and IL-1. Other compounds from the PGN are suggested to induce production of reactive oxygen species. DAP containing PGN is specifically correlated with increase cytotoxicity of lymphocytes and MDP is known to induce in the end the pro-inflammatory TNF-α and IL-1, but also chemokines, IL-8, CCL3 and CCL4, for recruiting cells that are responsible for pathogen clearance.[58]

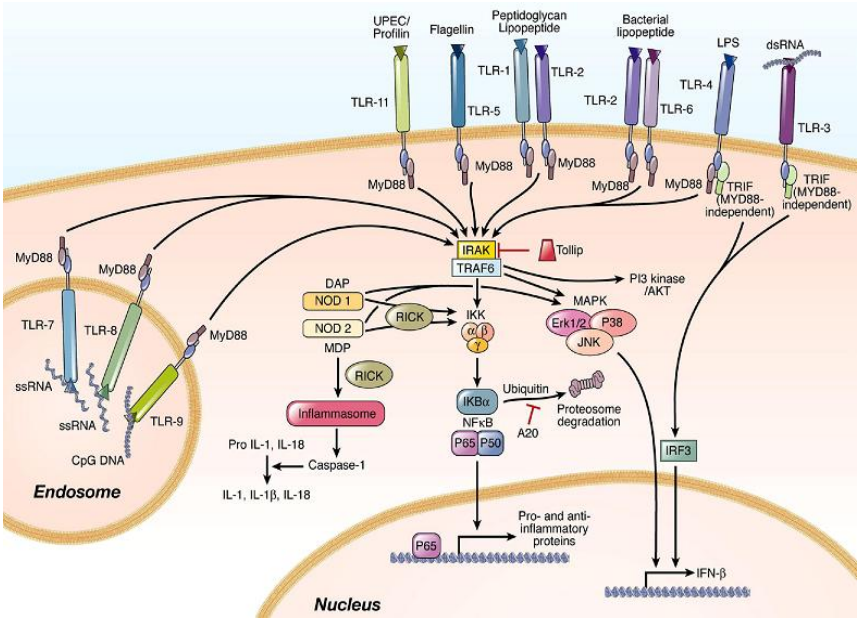

Figure 7. Recognition of bacterial ligands by membrane-bound toll-like receptors (TLR) and nod-like receptors (NLR), with signaling through conserved pathways such as NF-κB and mitogen-activated protein kinases (MAPK) signal transduction pathways.[72]

In summary, all knowledge about the several activated pathways, like JNK, ERK and NF-κB activation by Nod1 and Nod2 indicates that it remains difficult to point specific functions to one single receptor. But obviously, there is a large complex of activated intracellular pathways that are responsible for the production of pro and anti-inflammatory cytokines (Figure 7).

Conclusion

The previous parts showed an extended description of the peptidoglycan biosynthesis and the way it is recognized by our immune system when it enters our body. Bacteria use peptidoglycan in the first place for firmness against pressure from inside and protection from extreme environmental situations. Last decades, also the functional variety in structure has been described. The process of synthesis of peptidoglycan is investigated in detail by many researchers and more and more capabilities of bacteria using these varieties are known. In 1968 Schleifer and Kandler wrote a monumental work in which they classified different species of bacteria based on differential structures in the peptidoglycan.[23] But until today new structures are found, so we will never be able to map all structures. This is because bacteria are under constant pressure of new antibiotics, that kill bacteria by actions interfering with peptidoglycan, and this results in mutations that allow bacteria to survive in certain circumstances. Next to these antibiotics, also immune systems are a massive limitation for bacteria to survive. Also here bacteria are constantly change recognizable patterns of their cell wall to evade the immune system.

There are specific differences between gram positive and gram negative bacteria. These differences are seen back in the structure of their cell wall. When new species are found, structure determination identifies in almost all cases the origin of the bacteria. Some structures, the peptide stem for example, always show a typical composition that can be recognized, although often there are small alterations or modifications that are specific for that specie (Table 1). Modifications in the structure of peptidoglycan are often the result of different mechanisms during the PGN synthesis, but also after PGN synthesis modification are grown into the structural components, like acetylation, deacetylation and glycosylation of parts of the PGN backbone or peptide stem (Tabel 1).

Table 1. An overview of structural differences in parts of peptidoglycan. The table shows differences in amino acids in the peptide stem, cross-linking and backbone modifications in gram positive and gram negative bacteria.

Structure	**Gram positive bacteria**		**Gram negative bacteria**	
Peptide Stem	*standard*	*Variations*	*standard*	*variations*
Position 1	L-ala	gly, ser	L-ala	gly, ser
Position 2	D-glu	Amidation	D-glu	amidation
Position 3	L-lys		*meso*-DAP	Lanthionine, LL-DAP, cystathionine
Position 4	D-ala		D-ala	
Position 5	D-ala	D-lac, D-ser	D-ala	D-lac, D-ser
Cross-linkage	3-4 or 2-4 linkage		3-4 or 2-4 linkage	
Interpeptide	L-ala$_2$, L-ser-L-ala, gly$_5$, D-asx			
Anchored proteins	Iipopolysacchariden (LPS), Braun's Lipoprotein Murein lipoprotein (Lpp)		Wall Teichoic Acids (WTA) Lipoteichoic Acids (LTA)	
Backbone	*modification*			
Mur*N*Ac	O-Acetylation Phosphorylation *N*-Glycosylation			
Glc*N*Ac	*N*-Deacetylation			

As bacteria constantly change to evade immune systems, immune systems also evolve in different species to act more specific on pathogens. The human immune system has evolved in such a way that it has scanners that recognize specific patterns of pathogens. The so called pattern recognition receptors (PRR) are divided in different classes, based on the pathogen associated molecular pattern (PAMP) that they recognize and the response it initiates.

Recognition of the peptidoglycan layer in the cell wall of bacteria and its associated proteins are mainly recognized by NOD-receptors, TLRs and PGRPs.

PGRPs are first identified in insects, but these peptidoglycan recognition proteins are later also identified in mammals. Peptidoglycan recognition in certain parts in the human body is often correlated with antimicrobial peptide production and soluble PGRPs have been identified with amidase activity.
Toll-like receptors are well-studied immune receptors. Today, twelve different TLRs are known with their ligands. Toll like receptor 2, 4 and 6 are often related with the recognition of bacterial cell wall compounds. TLRs activate intracellular pathways that ultimately lead to activation of different transcription factors like NF-κB. The pathways that are activated are often mutually cross linked and the cytoplasmic domains of TLRs can also operate in a synergistic manner. TLR 2 alone is able to recognize the difference between gram-positive and –negative bacteria due to the high sensitivity to the DAP residues, but less to lysine-containing peptides from gram-positive bacteria. CD14 is also suspected of being responsible for the specific reaction to PGN residues, were it is activated by gram-positive bacteria, but also by LPS in gram-negative bacteria.[52]
This leads often to confusion in determining the effect of activation of certain TLRs. There are still controversial results about the activation of TLR by peptidoglycan. Some studies are convinced of the fact that TLR2 is specifically activated by LTAs anchored in gram-positive PGN fragments.[49]
Recent research showed that Nod activation by PGN also interferes with the TLR2 and TLR4 activation pathway that regulates antigen presentation. MyD88 knock-out mice are able to dim the TLR activations, which gave a clear image of the impact of Nod signaling pathway.[48]

For Nod receptors, recognition systems are divided by two variants of receptors in the Nod-like receptor family (NLR). Nod1 that mainly recognizes the *meso*-DAP, which is a fragment of the peptidoglycan. The N-terminal CARD domain of Nod1 then activates a pathway that results in NF-κB activation through RIP2 activation. Nod2, responsible for MDP recognition, also activates a NF-κB induced immune response. As told for TLR receptors, also Nod-like receptors to show interactions in their signaling pathways that cooperatively induce bacterial clearance. But the biggest difference between the two, is that Nod receptors act inside the cell in the cytoplasm, where TLR are membrane bound. Nod receptors belong to the Nod-like receptors that sense intracellular microbes and other danger signals and trigger several immune responses, including inflammasome activation and autophagy.[72] Nod signaling is tightly regulated after activation of a Nod receptor, were CARD domains start by binding to effector proteins and NF-κB pathways are activated but also caspase pathways are affected by CARD-CARD interactions.[69] Experiments also have shown interactions between the TLR and Nod pathways. Interactions early in the pathways are still unknown but end product synthesis is often influenced by the other. IL-1β is for example regulated by TRL activation, but Nod activation by PGN appears to downregulating this TLR-dependent IL-1β gene expression.[73]

Because of the general recognition patterns MDP and lysine containing residues for both TLR and Nod receptors it is hard to determine what receptor specifically activates transcription factors, as NF-κB, or other effector proteins. So far, a lot of research has been done into purified ligand exposure to several receptors. But also these studies provide controversial results, regarding to TLR2 activation by PGN or LTA.[49, 55]
Obviously, whole microbes contain both PAMPs for the Nod receptor and the TLRs. This means that receptors are activated at the same time. It is interesting to look at the synergistic effect between Nod and TLR. It has been showed before that LPS also activates Nod in combination with MDP residues. But recently also synergistic effects have been identified between Nod and TLR2, 5 and 7 by Mur-DAP residues.[74]

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