Antony van Leeuwenhoek was the first known person to lay eyes on the bacterial cell along with many other cells types. With his curiosity and superior lens grinding skills, he was able to create a microscope that could magnify specimens up to 200 times. At that time the compound microscope had already been invented nut could only magnify specimens 20 or 30 times larger. Van Leeuwenhoek’s first descriptions of bacteria, which he called animalcules, described the way they moved in water and that the water seemed to be alive. His perfection of magnification during that time and discovery of bacterial cells along with numerous types of other cells paved the way for many more discoveries involving microbiology (4). Bacterial cells are very different from our human cells. Some of the ways bacterial cells differ from or own cells include, they lack membrane bound organelles, have no nucleus, reproduce through conjugation, and have a cell wall. These are just a few differences between animal cells and bacterial cells there are many more. Our main focus in this chapter will be bacterial cell walls, specifically the peptidoglycan. We will be examining the structure, different types, function of bacterial cell wall along with the interaction bacterial cells wall components have with our immune cells.

Bacterial cell walls come in many different, shapes, sizes and compositions. That being said most bacterial families have a common feature composing their cell wall, peptidoglycan. The few that do not contain peptidoglycan in their cell wall are some of the halophilic (salt loving) bacteria. Peptidoglycan is arguable one of the most important components of the cell wall. It gives the cell wall its shape, rigidity, and enable the cell to resist intracellular pressure (2). Peptidoglycan is made up of two glycan chains which run parallel to each other, and peptide chains that link that glycan chains together (2) The glycan chain consist of repeating unit of the amino sugars, N-acetyl glutamate and N-acetylmuaramic acid. These two sugars are covalently bonded through the β 1.4. Glyosidic bond .The strand terminates with the amino sugar 1,6 anhydromuramic acid. The terminating sugar allows space for the insertion of other molecules that are important to the cells physiology. There is not much variation that occurs in the glycan part of peptidoglycan. One variation involves adding an acetyl group to the methanol group on the 6 prime carbon on the N acetylmuaramic acid subunit. Another modification of the N acetylmuaramic acid subunit is glycosylation of the amide group on the two prime carbon. This involves the one of the hydrogens on methanol group attached to the carbonyl part of the amide to be replaced with a hydroxyl group. The other modification occurs on the other sugar subunit, N acetyl glutamate. The acetyl group of the amide group replaced with two hydrogens. These modifications have immunological implications that will be discussed later in the chapter. (1) The two parallel glycan stands are connected by peptide chains, having 4 or 5 amino groups. The lactyl group on the N acetylmuaramic acid sugar is bonded by its carboxyl to the pentapeptide amine group. This bonding between these two groups is called a peptide bond and it is formed through the condensation reaction, meaning a water molecule is lost during this reaction. The amino acids in this peptide chain are L-Alanine, D – Glutamate, mesodiaminopimelic acid in gram negative, L-lysine in gram positive , terminating with either one or two D-alanines. The two amino acids on the third position are especially important in the peptidoglycan structure. This two amino acids permit the peptide chain to form peptide bonds with other peptide chains, creating the cell wall polymer peptidoglycan. This allows for the characteristic net like structure of peptidoglycan to form giving it structural integrity. (1) The peptide portion of peptidoglycan contains much variation. The two ways the peptide portion exist in peptidoglycan are with peptide subunits or with peptide subunits connected through interpeptide bridges and variation occurs in both of these. The subunit crosslinking is accomplished by amino acids having an additional amino group. The 2 amino acid are not the only amino acids with an additional amino group, L-ornithine, L,L Dimethlyphenylalanine, meso-2,6-diamino-3-hydroxy-β-pimelic acid or hydroxy lysine could potentially be in the third position facilitating the crosslinking of other peptide chains. However these are less common then the two previously mentioned amino acids. The third position for the crosslinking is the most common. However , in Corynebacterium insidiosum, the crosslinking occurs differently , the third position has an amino acid incapable of forming a crosslink due to the fact it only has one amino group which is already formed a peptide bond with the subsequent amino acid in its own peptide chain. The second positon is occupied by D glutamic acid. D glutamic acid has an additional carboxyl group, which allow the formation of the crosslink between it and another stem peptide.(2) Variation in the way crosslinks are formed are more abundant then variations in the peptide subunits. We will discuss many but not all the variations of this type. The most common crosslinking to adjacent peptide chains, which is known as group A cross linkage, is the crosslinking with the third and fourth position. This is known as a direct cross linkage where no interpeptide bridge is present. The amino acid mesodipimelic acid or L-lysine forms a peptide bond with the D-alanine that occupies the firth position in the adjacent peptide chain. Group A has three different subtypes involving different amino acids occupying the third position. The first subtype, A1 contains three variations on which different amino acids are in the third position of which have the capability of forming crosslink with adjacent peptide chains through an additional amine group.(2) The second subtype, A2 contains polymerized peptide subunits that facilitate the cross linkage. The peptide subunits are linked by a bridge of other peptide subunits. This peptide bridge can contain up to four peptide subunits. Subtype A2 has many unusual features. Half of the n-acetylmuramc acid subunits have not formed peptide ds with peptide chains and a more than half of them have no substitutions on L-lysine. A2 has no other variations. (2) The third subtype, A3 is very common in gram positive bacteria. This groups cross linkages occur through an interpeptide bridge containing monocarboxylic L amino acid, such as L-alanine, or glycine connecting the third and fourth position of adjacent peptide chains. The most common amino acid in the third position of a peptide chain in this group is l-lysine. The size of the interpeptide bridge is between 1 and 6 amino acids. The forth subtype, A4 contains crosslinks by interpeptide bridges that use amino acids with two carboxyl gropes, dicarboxylic amino acids.(2) The second type is , group b cross linkage, which is far less common than group A. Group B consists of cross linkages that occur at the second and forth positions of the adjacent peptide chain . This type of cross linkage only occurs in some of the coyrneform Bactria and involves the D-glutamic acid in the second position and the D-alanine in the forth position on the adjacent peptide chain. Group B has much variation in the peptide subunit of the second and forth positions .An interpeptide bridge that has an amino acid with two amino groups forms a peptide bond between the D-glutamic acid and D-alanine. This is done by the carbonyl group of the D-glutamic acid forming a bond between the amino groups of the interpeptide bridge. The remaining amino group of the interpeptide bridge forms a bond between itself and the carboxyl group on the D-alanine on the adjacent peptide chain. Group B has two subtypes which area dependent on the classification of the amino acid in the peptide bridge, whether it be L or D.(2) Peptidoglycan will contain three to six chemically different amino acids. Only certain amino acids are present in peptidoglycan. The amino acids valine, leucine and other branched amino acids are absent peptidoglycan. Aromatic, sulfur containing amino acids as well as histidine, arginine and proline have not be found in peptidoglycan either. D-glutamic acid in the second position and the D-alanines on the fourth and fifth positions are very rarely substituted by other amino acids. Positions 1 and 3 of the peptide chain have great variance in the amino acid present .In position one L-alanine, L-serine or glycine can be present. The third position can contain one of nine different amino acids. Differences in the distribution of the peptidoglycan type exist between the different groups of bacteria. (2)

### So far we have examined the glycan structure of peptidoglycan as well as the amino acid composition of the peptide chains. Now we will look at some of the different peptidoglycan composition of different groups of bacteria. Gram negative bacteria like, Pseudomonas, Nitrobacter, Salmonella and many others only contain the amino acids ,D-glutamic acid, L,D- alanine and mesodipimelic acid. There is not much variation in the gram negative distribution of peptidoglycan Some other features of gram negative bacteria are they have a thin layer of peptidoglycan in between an inner and outer membrane. Periplasmic space exists between the peptidoglycan and both the inner and outer membranes. The outer membrane contains lipopolysaccharides, which will be discussed later. Porins allow access to certain molecules entering or exiting the cell and are located on the outer membrane.(2)

### Gram positive bacteria exhibit extreme variation on the distribution and composition of peptidoglycan. The family Micrococcaceae contain many different. There are many genera in this family that have varying peptidoglycan structures. Genus Staphylococcus peptidoglycan contain a high amount of glycine. Staphylococcus *aureus* strains contain crosslinks of peptide chains that consist of 5 or 6 glycines. L-serine can replace the glycine in small amount in the interpeptide bridge. A substantial amount of L-serine can be seen if there is a large amount contained in the medium. The genus Micrococcus contains peptidoglycan structure that can be divided into two groups. Micrococcus *luteus* belongs to group A whichcontains peptidoglycan of group A subtype 2. The second group of this family, Micrococcus roseus and micrococcus *varians*, contain peptidoglycan of group A subtype 3. In this group the peptide chains are cross linked through the interpeptide bridge that contains 3 or 4 alanines. Interestingly these two groups appear o have correlation with the peptidoglycan type and the ability to transformate genetic information .Transformation involves the uptake of exogenous genetic material causing genetic alteration. For example Micrococcus roseus can only undergo this process with bacteria that have peptidoglycan of group A subtype 2. Two different strains of micrococcus *rosues* contain an interpeptide bridge that consists of tri-L-alanyl-L-threonine peptide instead of the normal peptide bridge containing tri or tetra alanine. (2)

### The family Lactobacillaceae bacteria genera are microaerophilic or anaerobic rods and cocci. There are seven genera of this class but we will just look at a few. The genus Streptococcus have 12 different types of amino acid composition in the peptidoglycan structure. The majority of the types contain interpeptide bridges containing glycine, L amino acids with one carboxylic group or both. They also contain L-lysine in the third position on the peptide chain. A few of them consist of crosslinks that are formed through an interpeptide bridge containing a dicarboxylic amino acid or a direct connection between the two adjacent peptide chains. The interpeptide bridge containing L-alanyl is present in many different streptococci.­­­­ In some other streptococci strains the interpeptide bridge contains L-serine or glycine instead of L-alanyl. (2)

Anaerobic streptococci that may be pathogenic or belong to normal flora. These streptococci belong to the genus Peptostreptococcus. Peptostreptococcus putridus has Lysine attached to D-aspartic acid in its peptidoglycan. Peptostreptococcus intermedius has peptidoglycan that contains lysine attached to L-alanine. (2) The Corynebacteriaceae family contains irregular rob shaped bacteria. The peptidoglycan structure in this family contains the greatest amount of variation when compared to other families if bacteria. 28 different peptidoglycan types exist and a little less than half belong to this family. The most common peptidoglycan type of Corynebacteriaceae has a direct crosslink of two adjacent peptide chains through the amino acid mesodipimelic acid. Facultative anaerobic Corynebacteriaceae contain this direct crosslink along with arabinogalactan. The genera Brevibacterium and Arthrobacter. Have the peptidoglycan type contains L-lysine in the third position of the peptide subunit. In the genus Cellulomonus all species with one exception, cellulomonas flavigena, contain the peptidoglycan type group A subtype 4 . This contains L-ornithine and D glutamic acid.(2) These are just some of the examples of the variations of the peptidoglycan structure. A few families were touched on to show how much the peptide portion of peptidoglycan can vary. We will now focus on how peptidoglycan interacts with our immune cells. When our immune cells recognize that a pathogen is present it is through binding of components of the bacterial cell wall to our immune cells. Peptidoglycan fragments are released during cell division, growth and lysis. Nucleotide oligomerization domain recognition proteins (NOD). NOD 1 recognizes the presence of a pathogen through the binding of the peptidoglycan fragment N acetylmuaramic acid connected the tripeptide, L-alanine, D-glutamic acid, and mesodipimelic acid .NOD 2 recognizes N acetylmuaramic acid with the tripeptide containing L-lysine or mesodipimelic acid. When the peptidoglycan fragment is recognized the innate immune system is activated through the transcription of specific genes ultimately leading to the production of cytokines. Cytokines are chemical messengers that attract immune cells to the site of the pathogen. Toll like receptors are on many immune cells such as dendritic cells and monocytes. These can recognize many bacterial cell wall fragments including peptidoglycan. Recognition by toll like receptors has the same effect of recognition by NOD 1 and 2. Peptidoglycan recognition proteins have the ability to directly kill the pathogen once it has been recognized. They interact with intact peptidoglycan and kill the bacteria through induction of membrane polarization and the production of free radicals .Lysozyme is a enzyme that causes the bacterial cell wall to lyse. It also interacts with intact peptidoglycan. This interaction cause the glyosidic bond between N acetylmuaramic acid and N acetyl glutamate to be cleaved leading to cell lysis. Bacterial cells have developed ways to evade our immune cells. This evasion is done through modifications of the peptidoglycan along with other components of the bacterial cell wall. Modification ­­of the glycan portion of peptidoglycan evade the lytic activity of lysozyme. Modification of the peptide portion of peptidoglycan evade recognition by our immune cells. As previously mentioned there are three modifications of the glycan portion. Those are O-acetylation of N acetylmuaramic acid, N-deacetylation of N acetyl glutamate and N glycosylation of N acetylmuaramic acid. These prevent lysozyme from binding, therefore preventing their lysis by lysozyme. Modifications of the stem peptide result in the bacteria not being recognized. Some modifications include substitution of the amino acid mesodipimelic acid with L-ornthinine. (3)

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