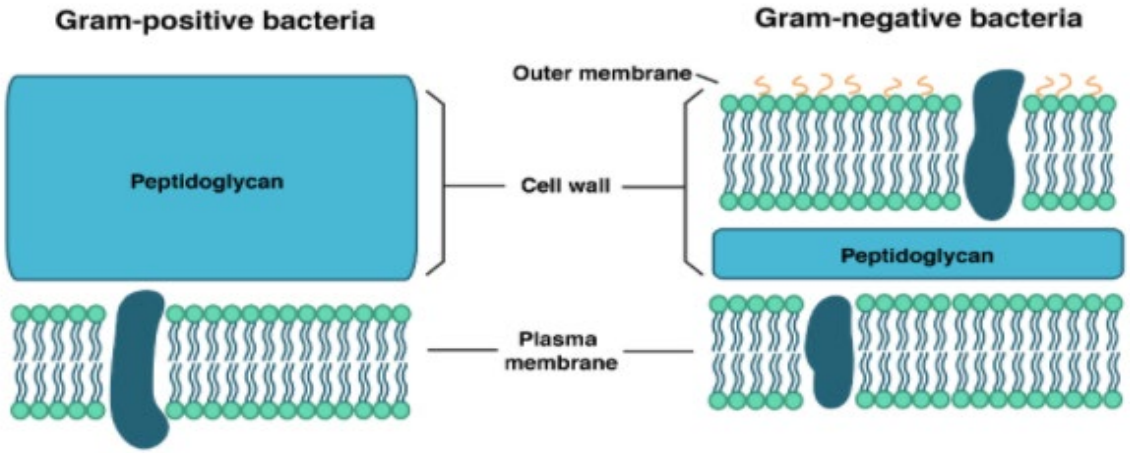
Bacterial Cell wall: Structure and Composition

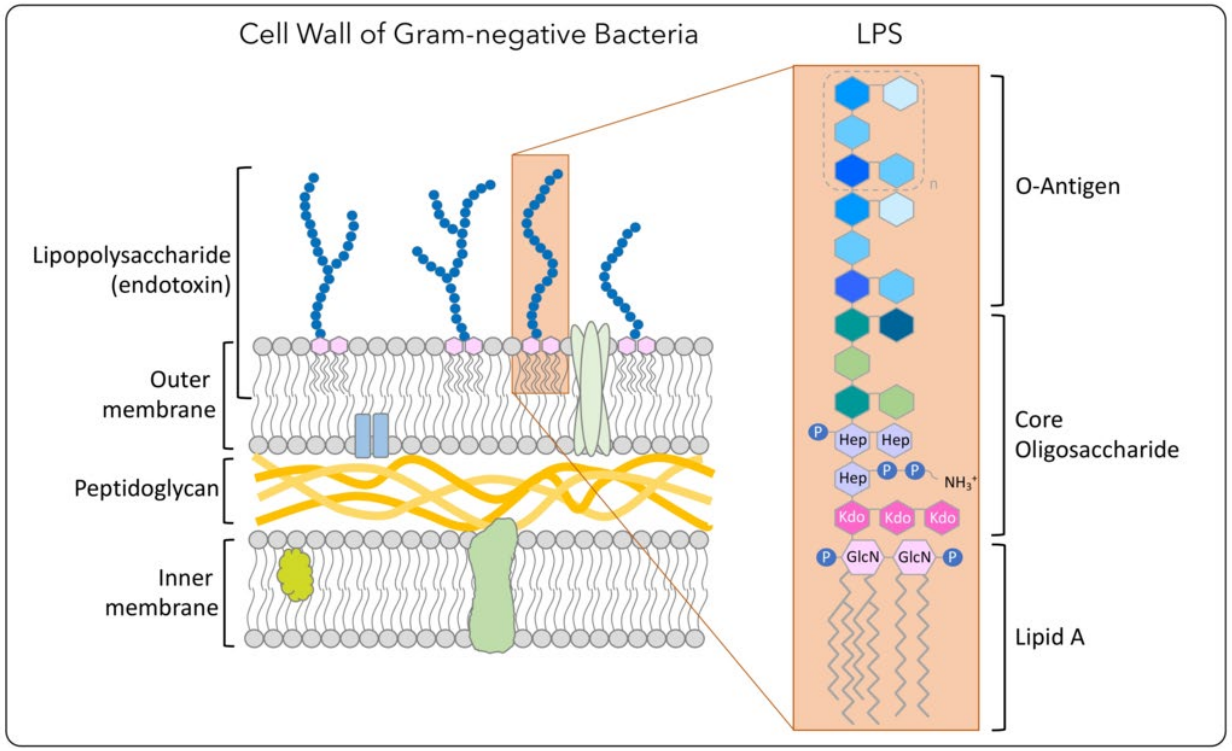
- Cell wall provides shape, rigidity and support to the cell.
- On the basis of cell wall composition, bacteria are classified into two major group;

Gram Positive and gram negative.



Parameter	Gram-positive bacteria	Gram-negative bacteria
Cell Wall	A single-layered, smooth cell wall	A double-layered, wavy cell-wall
Cell Wall thickness	The thickness of the cell wall is 20 to 80 nanometres	The thickness of the cell wall is 8 to 10 nanometres
Peptidoglycan Layer	It is a thick layer/ also can be multi-layered.	It is a thin layer/ often single-layered.
Teichoic acids	Teichoic acids are present.	Teichoic acids are not present.
Lipopolysaccharide	Lipopolysaccharide is not present.	Lipopolysaccharide is present.
Outer membrane	The outer membrane is not present.	The outer membrane is mostly present.
Lipid content	The Lipid content is very low.	The Lipid content is 20% to 30%.
Resistance to Antibiotic	These are very susceptible to antibiotics.	These are very resistant to antibiotics.

Endotoxin: lipopolysaccharides (LPS)



- LPS is the major component of the outer membrane of Gram-negative bacteria.
- They do not retain crystal violet as they carry a thin cell wall which is made of a single layer of peptidoglycan surrounded by the outer membrane which contains LPS.
- Endotoxins serve as an early diagnostic biomarker to serologically identify Gram-negative-specific bacterial infections. Timely identification for early disease treatment.
- Endotoxin binds to host macrophages and induces release of pro-inflammatory cytokines and excessive inflammation can lead to multiple organ failure and death.

Peptidoglycan: Consist of glycan (sugar) backbone with crosslinked peptide side chains. It gives support against osmotic pressure, it is **site of action of penicillin**. Penicillin interferes with the production of peptidoglycan, makes cell leaky and fragile.

Lipid A: Acts as anchor found in outer membrane of gram negative bacteria. Toxic component of endotoxin

Teichoic acid: Major surface antigen. They are negatively charged polyol phosphate polymers, covalently linked with peptidoglycan.

Peptidoglycan, also known as murein:

•Peptidoglycan is porous cross linked polymer which is responsible for strength of cell wall.

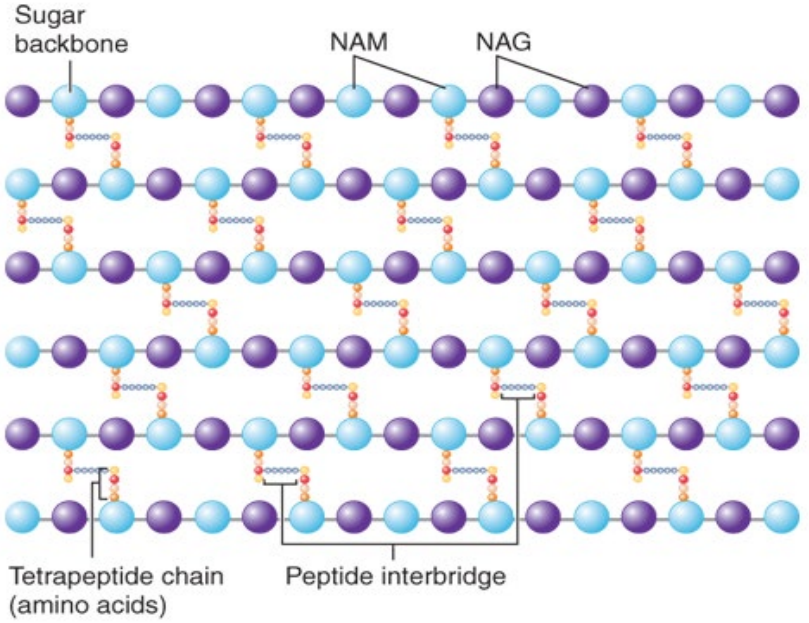
•Peptidoglycan is composed of three components.

- **Glycan backbone**
- **Tetra-peptide** side chain (chain of 4 amino acids) linked to NAM
- **Peptide cross linkage**

•Glycan backbone is the repeated unit of NAM and NAG) linked by β-glycosidic bond.

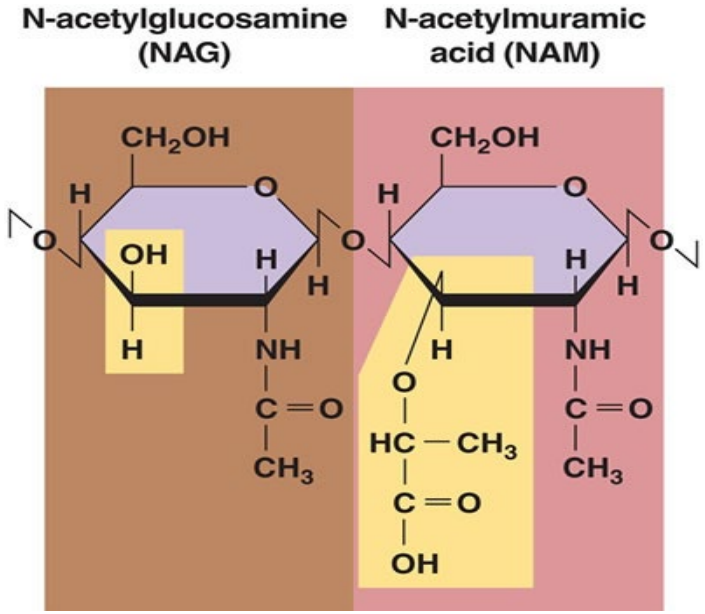
•More than 100 peptidoglycan are known with the diversity in chemistry of peptide cross linkage and interbridge.

•Although the peptidoglycan chemistry vary from organism to organism the glycan backbone ie NAG-NAM is same in all species of bacteria.

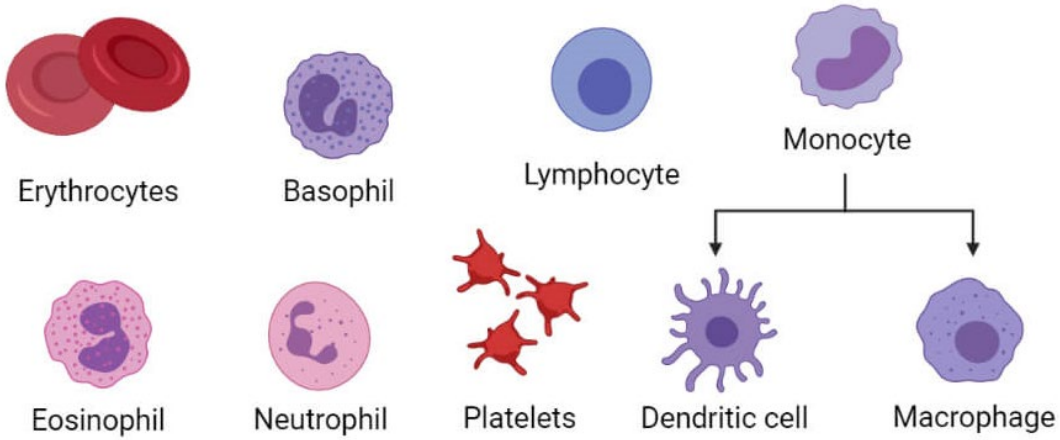


The aminoacids found in tetra-peptide are-

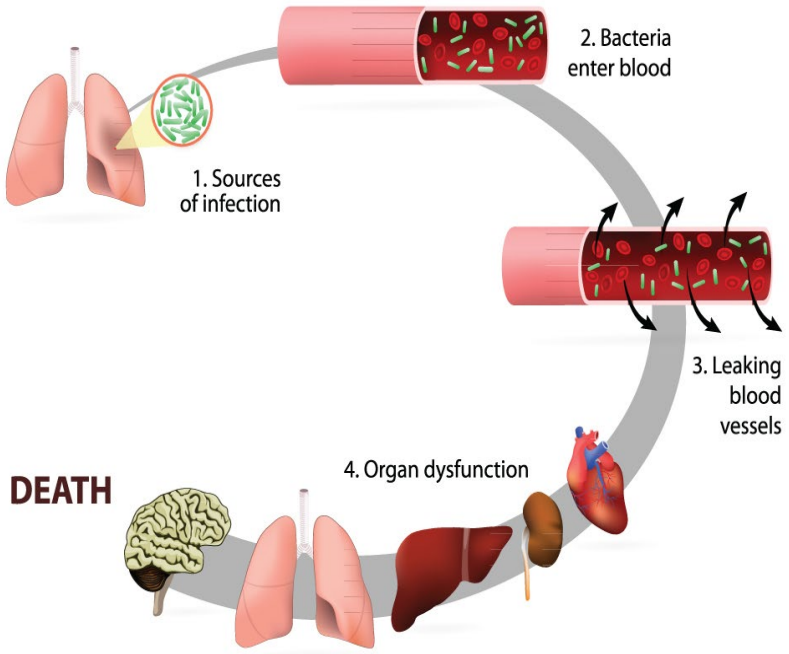
- **L-alanine:** 1st position in both gm+ve and gm-ve bacteria
- **D-glutamic acid:** 2nd position
- **D-aminopimelic acid/ L-lysine:** 3rd position (variation occurs)
- **D-alanine**: 4th position



Sepsis is a life-threatening illness caused by body's response to an infection. Immune system protects from many illnesses and infections, but it can also be over-activated in response to an infection



Macrophages are specialised cells involved in phagocytosis and destruction of bacteria and other harmful organisms.



nature medicine

Extracellular histones are major mediators of death in sepsis

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Hyperinflammatory responses can lead to a variety of diseases, including sepsis[1]. We now report that extracellular histones released in response to inflammatory challenge contribute to endothelial dysfunction, organ failure and death during sepsis. They can be targeted pharmacologically by antibody to histone or by activated protein C (APC). Antibody to histone reduced the mortality of mice in lipopolysaccharide (LPS), tumor necrosis factor (TNF) or cecal ligation and puncture models of sepsis. Extracellular histones are cytotoxic toward endothelium *in vitro* and are lethal in mice. *In vivo*, histone administration resulted in neutrophil margination, vacuolated endothelium, intra-alveolar hemorrhage and macro- and microvascular thrombosis. We detected histone in the circulation of baboons challenged with *Escherichia coli*, and the increase in histone levels was accompanied by the onset of renal dysfunction. APC cleaves histones and reduces their cytotoxicity. Co-infusion of APC with *E. coli* in baboons or histones in mice prevented lethality. Blockade of protein C activation exacerbated sublethal LPS challenge into lethality, which was reversed by treatment with antibody to histone. We conclude

(**Supplementary Fig. 1b**). Sequencing identified the 10-kDa protein as the mouse histone H4 (H4) internal sequence methyl-Lys20–Ile34. The 13-kDa protein matches the mouse histone H3 (H3) internal sequence Lys27–Lys36. The N-terminal sequence of the 15-kDa band protein could not be determined by direct Edman sequencing. After in-gel tryptic digestion, tandem mass spectrometry identified three peptide sequences that match the mouse histone H2A protein sequences Ala21–Arg29, His82–Arg88 and Val100–Lys118. These data suggested that extracellular histones are cytotoxic toward endothelium and that APC is cytoprotective because it cleaves them. We confirmed the H3 identification by western blotting with antibody to H3 (**Supplementary Fig. 1c**). The apparent increase in histone fragments in the conditioned medium of activated macrophages cultured with APC might indicate that APC can cleave not only the soluble extracellular histones in the medium but also the histones associated with the activated cells or DNA.

To determine whether histones are toxic to endothelium and whether APC can lower the histone cytotoxicity, we treated EA.hy926 cells with a mixture of histones or five individual histones. We found that a mixture of histones was cytotoxic to these