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Bacterial Virulence
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ADHESION

Lecture one

BY
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Definitions

Virulence: The quantitative ability of an agent to cause disease. Virulent agents cause disease when introduced into the host in small numbers. Virulence involves adherence, invasion, and toxigenicity.

Adherence (adhesion, attachment): The process by which bacteria stick to the surfaces of host cells. After bacteria have entered the body, adherence is a major initial step in the infection process.

Invasion: The process whereby bacteria, animal parasites, fungi, and viruses enter host cells or tissues and spread in the body.

Toxigenicity: The ability of a microorganism to produce a toxin that contributes to the development of disease.

Adhesion

Adhesins are bacterial components that mediate interaction between the bacterium and the host cell surface. Cell adhesion molecules play important structural roles and are involved in various signal transduction processes. As an initial step in the infectious process, many bacterial pathogens adhere to cell adhesion molecules as a means of exploiting the underlying signalling pathway, entering into host cells or establishing extracellular persistence. Often, bacteria are able to bind to cell adhesion molecules by mimicking or acting in place of host cell receptors or their ligands.

Bacterial engagement of host cell receptors can be a means of targeting a pathogen to a particular niche, co-opting underlying signaling pathways, establishing persistent infections and inducing invasion. Invasion affords bacteria protection from immune detection and facilitates access to deeper tissues.

Many bacterial pathogens have evolved the capacity to adhere to cell adhesion molecules (CAMs). **CAMs are cell-surface receptors that**

mediate cell to cell and cell to extracellular matrix (ECM) interactions. Generally, they can be classified into four main groups: **integrins, cadherins, members of the immunoglobulin superfamily of CAMs (IgCAMs), and selectins**. Bacteria are able to bind to CAMs by mimicking or acting in place of host cell receptors or their ligands.

Integrins

Integrins are heterodimeric transmembrane proteins, made up of non-covalently paired α and β subunits, which serve as **adhesion and signalling** hubs at the cell surface. Once bound to its ligand, an integrin not only provides adhesion, but also initiates signalling mechanisms which allow cells to respond to the mechanical and chemical properties of the cellular microenvironment.

Integrins have been shown to interact with various proteins on the surfaces of eukaryotic, prokaryotic, and fungal cells, as well as a range of viruses. Typically, ligand binding is carried out through integrin receptor recognition of small peptide sequences. Target sequences for integrins can be as simple as the **RGD** or **LDV** tripeptides, or more complex as in the case of the **GFOGER** peptide (where O stands for hydroxyproline). Many classical ECM proteins contain these short integrin recognition motifs. **RGD** sequences are found in both vitronectin and fibronectin, an **LDV** motif is present in fibronectin, **GFOGER** is found within collagen.

Many bacteria take advantage of the binding capabilities of integrins on cell membranes for infectious purposes. Some bacteria utilize specific integrin dimers for cellular binding, while others exploit extracellular fibrous proteins that naturally bind to integrins for the purpose of translocating virulence factors.

It has been demonstrated that association of fibronectin binding *S. aureus* with host cell β1 integrins leads to integrin clustering and activation,

which in turn triggers characteristic host cell signaling pathways and the accumulation of a focal adhesion like protein complex in the vicinity of attached *S. aureus*. In this regard, several well characterized integrin-associated proteins, e.g. vinculin, paxillin, zyxin, tensin, FAK and c-Src are recruited upon binding of *S. aureus* to host cells and a re-organization of the actin cytoskeleton is observed.

It has been proposed that *S. aureus* attachment sites are reminiscent of focal adhesion- and fibrillar adhesion-like structures, which under physiological conditions provide a connection from ligand-bound integrins, via intracellular integrin-associated proteins, to the actin stress fibers. By actin-myosin contraction, cells can exert pulling forces on their integrin-based adhesions and this could provide a compelling mechanistic explanation for the integrin-mediated uptake of particles.

Cadherins

Cadherins (cad) belong to the superfamily of **CAMs**. Characterized by their adhesion properties mediated through repeated extracellular cad domains (ECs) under Ca^{2+} control, cad play a key role in cell-to-cell interactions. Several subtypes of cad are encoded in the human genome. These molecules were classified according to their tissue distribution: for example, the P prefix is used in P-cad (encoded by *CDH3*) to define placental cad, N-cad (encoded by *CDH2* and *CDH12*) for neural cad, VE-cad (encoded by *CDH5*) for vascular endothelial cad, and E-cadherin (E-cad; encoded by *CDH1*) for epithelial cad.

Many bacterial species use cad as target receptors. *Fusobacterium nucleatum*, a pathogen associated with oral plaque formation and colorectal cancers, binds E-cad through its FadA adhesin. *Listeria monocytogenes*, the causative agent of severe food poisoning, which sometimes lead to meningitis, internalizes when internalin A (InlA) and

InlB bind to E-cad and the hepatocyte growth factor receptor on the basolateral surface of epithelial cells. *Helicobacter pylori*, a bacterium responsible for severe gastric disease, adhere to target cells through interaction with carcinoembryonic antigen-related CAM (CEACAM) cell-surface receptor *via* its HopQ adhesin. Then, the bacterial HtrA sheddase cleaves the gastric epithelial cell-to-cell junctions through endoproteolysis of E-cad, occludin, and claudin-8. After transmigration of *H. pylori* to the basolateral membrane of gastric epithelial cells, the T4SS pilus is activated and injects the CagA cytotoxin into the target cell where the release of β-cat is stimulated.

Immunoglobulin superfamily of CAMs

The immunoglobulin superfamily (IgSF) is a large protein superfamily of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. Molecules are categorized as members of this superfamily based on shared structural features with immunoglobulins, also known as antibodies. They all possess a domain known as an immunoglobulin domain or fold, which is a type of protein domain that consists of a 2-layer sandwich of 7-9 antiparallel β-strands arranged in two β-sheets with a Greek key topology, consisting of about 125 amino acids. Members of the IgSF include cell surface antigen receptors, co-receptors and co-stimulatory molecules of the immune system, molecules involved in antigen presentation to lymphocytes, cell adhesion molecules, certain cytokine receptors and intracellular muscle proteins (Figure 1).

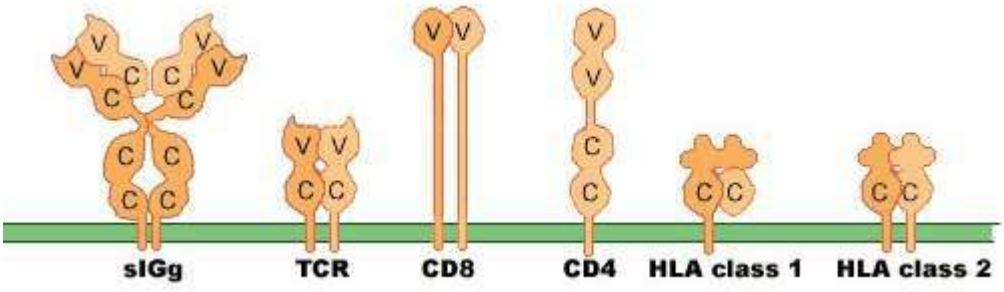

Figure 1: Immunoglobulin superfamily

Neisseria gonorrhoeae is a human-specific pathogen that infects mucosal tissues of the genitourinary tract. Initial attachment to mucosal surfaces is mediated by neisserial type-IV pili. Subsequently, intimate adherence is conferred by colony opacity-associated (Opa) proteins, a family of phase-variable outer-membrane proteins that act as adhesins and facilitate invasion. A single gonococcal strain can possess up to 11 different Opa variants. A few Opa variants bind heparan sulfate proteoglycans on epithelial cells or use vitronectin and fibrinogen to bind indirectly to integrins. Most Opa proteins target carcinoembryonic antigen-related CAM (CEACAM) receptors, which are members of the IgCAMs and are widely distributed throughout human host tissues. The function of CEACAM receptors is not completely understood; however, it is known that they mediate cell—cell adhesion and play a role in cell cycle control and cell differentiation.

Neisseria gonorrhoeae specifically targets four CEACAM family members: CEACAM 1, -3, -5 and -6. In human epithelial cells, engagement of CEACAM receptors by Opa proteins results in bacterial engulfment. In the polarized epithelial cell line T84, Opa-mediated binding to CEACAMI, -5 or -6 induces invasion, transcytosis and release of bacteria at the basolateral surface. In neutrophils, Opa proteins mediate adherence and opsonin independent phagocytosis when targeted to

CEACAM 1, -3 and -6 receptors. In certain cases, *N. gonorrhoeae* is capable of triggering expression of its own cellular receptor. CEACAM 1 expression is upregulated in endothelial cells in an NF kB-dependent manner in response to gonococcal infection or purified lipopolysaccharide. Consequently, this probably reinforces Opa-mediated binding and invasion.

Interestingly, recent studies have shown that the engagement of different CEACAM receptors by *N. gonorrhoeae* results in distinct signaling consequences and mechanisms of entry (Figure 2). Adherence and invasion of gonococci using CEACAM1 or -6 results in actin- and Rho-GTPase-independent pseudopod formation. Conversely, invasion via CEACAM3 induces actin-driven membrane projections involving Rac1 and Cdc42. The neutrophil-restricted CEACAM3 receptor has an immunoreceptor tyrosine-based activation motif (ITAM) encoded in its cytoplasmic domain. ITAM motifs are associated with signaling activation of T-cell, B-cell and Fc receptors (FcRs). In fact, engagement of CEACAM3 is quite similar to engagement of FcR (Figure 3).

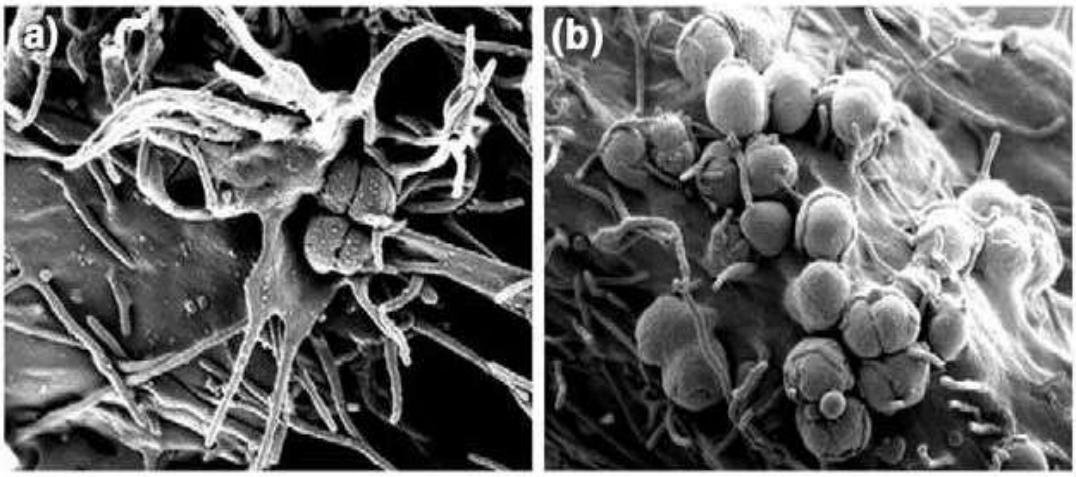

Figure 2: *Neisseria gonorrhoeae* invasion. (a) CEACAM3-mediated invasion induces impressive, extended surface projections at the site of adherence. (b) CEACAM1 and -6 (shown) invasion results in the tight envelopment of bacteria within pseudopods.

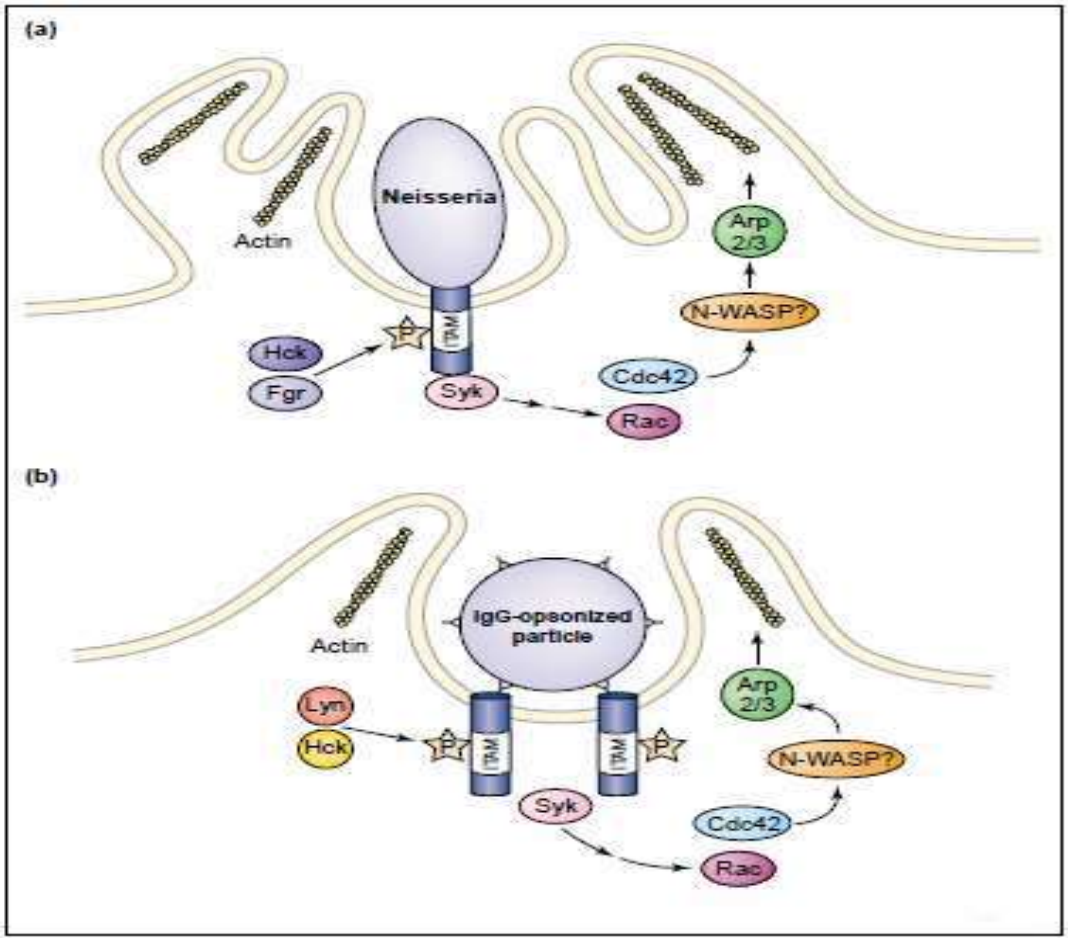

Figure 3: Comparison of the signaling pathways involved in (a) CEACAM3-mediated invasion by N. gonorrhoeae and (b) FcR-mediated phagocytosis. Src-family kinases (Hck and Fgr in [a] and Lyn and Hck in [b]) phosphorylate tyrosine residues within the receptor's ITAM motif. Syk tyrosine kinase is recruited to the phosphorylated ITAM motif, where it is activated. Syk then transmits a downstream signal that leads to the activation of Rac and Cdc42, which might activate N-WASP, leading to actin polymerization via the Arp2/3 complex. IgG, immunoglobulin G; N-WASP, neuronal Wiskott–Aldrich syndrome protein; P, phosphate.

Selectins

Selectins are Ca^{2+}-dependent lectins, consisting of three subfamily members (P-, E-, and L-selectins in platelets, endothelial cells, and leukocytes, respectively) that all contain an N-terminal C-type domain. Structurally diverse glycoprotein ligands bind to selectins with high affinity.

The best-characterized adhesin of *H. pylori* is BabA, which binds Lewis B (Le^b), a fucosylated histo-blood group antigen present on red

blood cells and in the gastrointestinal mucosa. Recently, a second receptor–adhesin interaction was identified. When gastric biopsy tissue from *H. pylori*-infected individuals was infected with a babA mutant, adherence was still observed. Moreover, binding of the babA mutant was not blocked by pretreatment of bacteria with soluble Le^b antigen. This suggests there is another adhesin, possibly specific to inflamed (i.e. *H. pylori*-infected) tissue, that binds a receptor distinct from that of BabA. Using thin layer chromatography, mass spectrometry and nuclear magnetic resonance, sialyl-dimeric-Lewis x (Le^x) glysophospholipid was identified as a second receptor. This was confirmed using competitive inhibition and monoclonal antibody inhibition studies. Using a retagging method, this sialyl-dimeric-Lex-binding bacterial adhesin was identified as the bacterial outer membrane protein SabA.

Sialylated glycoconjugates are low in healthy gastric mucosa but are expressed during gastritis. Upon inflammation, sialyl-Le^x serves as a receptor for selectins and mediates the initial steps of leukocyte migration through the endothelium. Recent studies demonstrate that *H. pylori* infection stimulates expression of sialyl-Le^x at the surface of the gastric mucosa. *H. pylori* is therefore another example of a bacterial pathogen capable of inducing expression of its own receptor, which can then be exploited for further adherence. Using selectin mimicry, *H. pylori* can take advantage of the host's own mechanism by which it initiates an immune response to infection. By specifically targeting sites of inflammation, *H. pylori* might benefit nutritionally from exudates released upon cell damage and could take advantage of the exposed ECM components for further colonization. Adherence to sialylated Le^x expressed during chronic inflammation might therefore contribute to the exceptional ability of *H. pylori* to establish persistent infections.

CAPSULAR POLYSACCHARIDE AND THEIR ROLE IN VIRULENCE

Lecture two

BY

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Introduction

Bacterial pathogens exhibit a number of virulence factors that enable them to invade and colonize the tissues of host organisms. A number of these virulence factors are displayed on the cell surface and include adhesins that mediate attachment to host cells, toxins that may be secreted resulting in host tissue damage, and the possession of molecules that render them resistant to host antimicrobial defences. Capsular polysaccharide (CPS) has long been recognized as an important virulence determinant in isolates capable of causing infection in humans and animals. CPS is found on the outermost surface of a wide range of the bacteria and may be linked to the cell surface via covalent attachments to phospholipid or lipid A molecules. In contrast, extracellular polysaccharide (EPS) molecules appear to be released onto the cell surface with no visible means of attachment. Such EPS can be loosely associated with the cell surface and easily sloughed off as slime.

CPS molecules are highly hydrated and typically constitute more than 95% water. They are composed of repeating single monosaccharide units that are joined by glycosidic linkages. CPS may be homo- or heteropolymers and can be substituted with both organic molecules such as acetyl groups, and inorganic molecules such as phosphate. In addition, two monosaccharides may be joined in a number of configurations due to the presence of multiple hydroxyl groups within each monosaccharide that may be involved in the glycosidic linkage. Thus, CPS are a diverse range of molecules that can differ not only in their constituent monosaccharides but also in the manner in which they are joined. This diversity is illustrated in bacterial species such as *Escherichia coli* where over 80 distinct capsular

serotypes have been described while in *Streptococcus pneumoniae*, there are over 90 capsular serotypes. The introduction of branches and substitution with organic or inorganic molecules to polysaccharide chains adds a further layer of structural complexity. However, chemically identical CPS may also be synthesised by different bacterial species. The group B capsule of *Neisseria meningitidis*, a homopolymer of a 2,8-linked N-acetylneuraminic acid (NeuNAc), is identical to the KI antigen of *E. coli*, while the CPS of *Pasteurella multocida* type D is identical to the *E. coli* K5 capsule which comprises repeating disaccharides of glucuronic acid linked to N-acetylglucosamine. The apparent conservation of particular CPS structures between taxonomically diverse genera of bacterial species raises intriguing questions regarding the evolution of capsule diversity and the acquisition of capsule biosynthesis genes.

Functions of Bacterial Capsules

As the polysaccharide capsule represents the outermost layer of the bacterial cell, it is not surprising that the capsule mediates interactions between the bacterium and its immediate environment. Accordingly, a number of functions have been ascribed to bacterial capsules. Each of these is directly relevant to pathogenicity and as such contributes to the role of CPS as a virulence factor:

1- Resistance to Desiccation

As CPS are highly hydrated molecules that surround the cell surface, they may protect bacteria from the harmful effects of desiccation. This property is probably most relevant in the transmission and survival of

encapsulated bacteria in the environment demonstrated in the cases of isolates of *E. coli, Acinetobacter calcoaceticus* and *Erwinia stewartii,* which have been shown to be more resistant to desiccation than their isogenic acapsular mutants. Furthermore, the capsule probably provides protection during transmission from host to host. In the case of *E. coli,* genes encoding enzymes for the biosynthesis of capsular colanic acid have been shown to be upregulated in response to desiccation. While the mechanism of regulation is unclear, it is thought that external osmolarity is altered during desiccation, and it has been shown that expression of alginate EPS of *Pseudomonas aeruginosa* as well as expression of the Vi CPS of *Salmonella enterica serovar* Typhi, which is essential for virulence, are increased in response to high osmolarity.

2- Adherence

CPS may mediate adhesion of bacteria to surfaces (both biotic and abiotic) and to each other. Adhesion to abiotic surfaces may result in the establishment of biofilms and EPS-mediated interspecies co-aggregation within biofilms can enhance colonization of various ecological niches. In addition, growth of bacteria as a biofilm may offer some protection from phagocytic protozoa and present nutritional advantages, while it is thought that the presence of EPS acts as a permeability barrier against antimicrobial agents. While adhesion to host tissues is undoubtedly a multifactorial process involving an array of bacterial surface components, CPS has been implicated in the adhesion of a number of human pathogens to host tissues. *Streptococcus pyogenes* or group A *Streptococcus* (GAS) is responsible for a range of clinical infections including skin infections, acute rheumatic fever,

streptococcal pharyngitis, streptococcal toxic shock syndrome and necrotizing fasciitis. In the development of pharyngitis, colonization of the pharynx by streptococci not only represents a vital stage in the life cycle of GAS, but it is also likely that that the pharynx serves as a reservoir for infection from which GAS may be disseminated to other hosts as well as causing invasive infections such as necrotizing fasciitis.

It has been demonstrated that the hyaluronic acid capsule of GAS binds to CD44 molecules on the surface of human keratinocytes, the predominant cell type in skin and the pharyngeal epithelium. Once bound, bacterial contact with the epithelial surface induces lamellipodia formation on the surface of keratinocytes (figure 1), which is not observed in an isogenic acapsular mutant. Gram-negative pathogens such as *Salmonella* and *Shigella* spp. also induce lamellipodia formation following binding to host epithelial cells; however subsequent fusion of the lamellipodia entraps the bacteria, resulting in their internalization. GAS are inefficiently internalized as a consequence of the possession of their hyaluronic acid capsule. Furthermore, the binding of GAS to CD44 induces marked cytoskeletal rearrangements and cell signalling events leading to the opening of intercellular junctions, which is thought to promote tissue penetration by GAS (fig. 1). Clearly this is not the case for all encapsulated pathogens, as the case of GAS involves molecular mimicry, with the CPS being identical to host hyaluronic acid. In other pathogens, initial attachment to host cells has been shown to be inhibited by encapsulation, as is the case for binding of *Klebsiella pneumoniae* to epithelial cell lines in vitro. Paradoxically, encapsulated isolates of the same strain adhered better to a mucus-producing cell line than an acapsular mutant. These data suggest that in some cases the CPS may

promote initial colonization of the mucus layer, while subsequent interaction with the underlying epithelial layer is reduced by the presence of a capsule, presumably due to the masking of bacterial components required for specific interaction with the epithelial surface. These observations support the notion that there is some form of co-ordinate regulation of capsule expression during the early stages of infection.

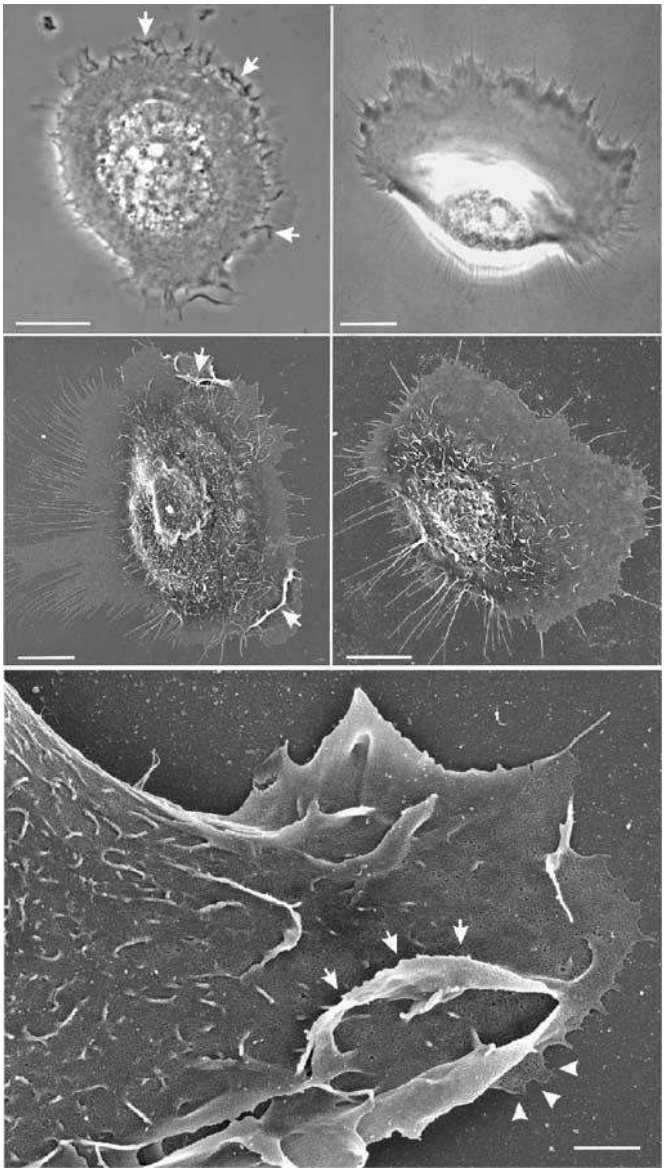

Figure 1: Lamellipodia of keratinocytes

Adherence and capsule production seem to be inversely regulated, with adherence being maximal at times when capsule production is lessened. This could be expected since a thick capsule would effectively mask adherence proteins on the cellular surface

3- Resistance to Non-Specific Host Immunity

During invasive infections of humans and animals by encapsulated pathogens, interactions between the bacterial CPS and immune system of the host play a critical role in determining the fate of the infection. During an innate host response, the bacterial capsule may confer some resistance to complement-mediated killing. The main function of the complement system is the binding of host peptides to foreign organisms. Once bound, these are recognized by specific complement receptors on host phagocytes that facilitate opsonization and subsequent destruction. Thus, activation of the complement cascade involves an array of serum and cell surface proteins and three pathways of activation are recognized:

1. In the classical pathway, an antibody response is generated,
2. While the alternative pathway can be activated in the absence of specific antigen-antibody recognition.
3. The mannan-binding lectin pathway recognizes surface polysaccharides and then activates the complement cascade.

These pathways generate C3 convertases that cleave C3 (the major complement component) to C3b, which can then bind to the cell surface.

Factor C3b and its degradation product iC3b are the primary complement opsonins. In the absence of specific antibody, CPS is thought to activate the alternative pathway in which C3b binds non-specifically to the bacterial surface. Bound C3b is then activated by interaction with factor B and forms the C3 convertase C3bBb, which binds to the bacterial surface along with further C3b. This complex termed C3b2Bb acts as the C5 convertase and promotes formation of the membrane attack complex (MAC), which can form pores in certain bacteria, causing their destruction.

CPS that contain *N*-acetylneuraminic acid (NeuNAc) are known to be poor activators of the alternative pathway and it is thought that this is because NeuNAc binds directly to factor H. Factor H promotes the binding of factor I to C3b, forming iC3b, which breaks the amplification loop of the cascade, which in turn prevents formation of the MAC. In such cases, the bacterial capsule usually acts in concert with other surface structures such as the O-antigen of lipopolysaccharide to confer resistance to complement-mediated killing. Thus, a particular combination of surface structures can confer a high degree of resistance to the innate immune response. In the case of other encapsulated pathogens, it is thought that the presence of a CPS may actually provide a barrier to complement components by physically masking underlying surface structures that would normally be potent activators of the alternative pathway.

Finally, CPS may confer resistance to complement-mediated opsonophagocytosis. In the case of *Staphylococcus aureus,* the presence of a thick capsule has been shown to be antiphagocytic, as it interfered with recognition of cell-bound C3b and iC3b by phagocytic receptors. Similar

observations have been made in the case of S. *pneumoniae* where CPS also appears to block cell-bound C3b (figure 2).

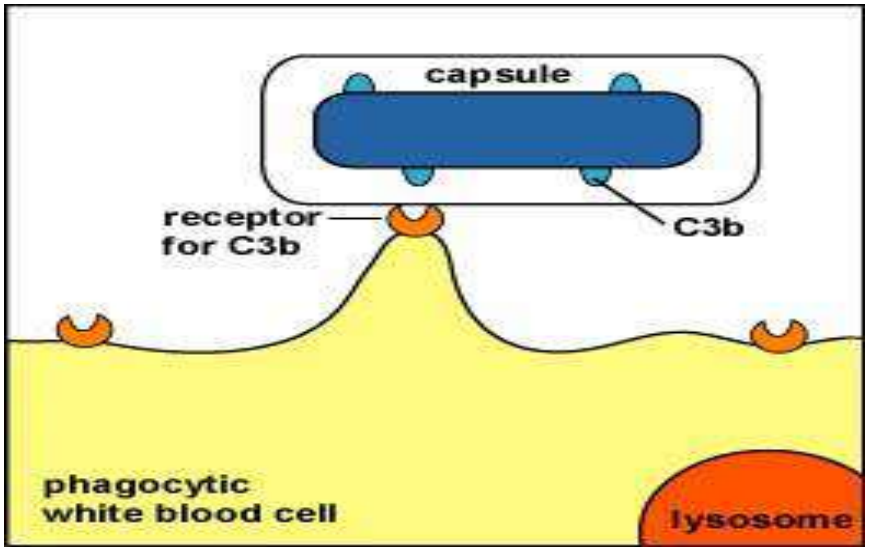

Figure 2: Inhibition of phagocytosis by capsule.

Furthermore, many CPS are highly negatively charged molecules and may also confer resistance to phagocytosis. In addition to these direct interactions between CPS and components of the complement system, certain CPS may modulate the host's immune system by stimulating the release of certain cytokines resulting in the disruption of the cell-mediated immune response. One such example is the CPS of *K. pneumoniae,* which was shown to induce high levels of interleukin-10 (IL-10) in experimentally infected mice, in contrast to an acapsular mutant. High levels of lL-I0 inhibit gamma interferon-induced activation of macrophages.

4- Resistance to Specific Host Immunity

Although many CPS elicit a specific (antibody-mediated) immune response in the host, a certain small set of CPS are able to confer some resistance. Capsules such as those that contain NeuNAc, e.g. *E. coli* KI and *N. meningitidis* serogroup B in addition to the *E. coli* K5 polysaccharide which is identical to N-acetyl heparosan (precursor in heparin/heparan sulfate biosynthesis), are poorly immunogenic. Infected individuals only mount a poor immune response to these antigens as a consequence of the structural similarities of these capsules to host polysaccharides encountered abundantly in the extracellular matrices. As a result, the expression of these capsules that mimic host structures provides protection against the specific arm of the host's immune response.

LECTURE THREE: ANTIMICROBIAL RESISTANCE AND VIRULENCE

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Antibiotic resistance and fitness cost

Antibiotic-resistant bacteria impose a substantial burden on the human population. In addition to morbidity and mortality caused by infections with resistant pathogens. In an environment that contains an antibiotic, possession of a corresponding resistance genes is clearly beneficial to a bacterium. However, in the absence of antibiotic, resistant genotypes may have lower growth rates than their sensitive counterparts**.**

Mutations that confer resistance do so by disrupting some normal physiological process in the cell, thereby causing detrimental side-effects. In the case of plasmid encoded resistance functions, bacteria must synthesize additional nucleic acids and proteins; this synthesis imposes an energetic burden and the products that are synthesized may interfere with the cell's physiology.

Evolving antibiotic resistance by horizontal gene transfer or by chromosomal mutation is associated with a fitness cost, as demonstrated by the fact that resistant bacteria have reduced growth rates and competitive ability relative to sensitive strains in the absence of antibiotics. Many studies have shown that resistant genotypes are less fit than their sensitive progenitors when the two compete in an antibiotic free medium. Some of these studies have demonstrated costs associated with carriage of plasmids and expression of plasmid encoded resistance functions whereas others have demonstrated side-effects of resistance mutations that impair growth.

Resistance to β-Lactams and Impact on Virulence

β -Lactam antibiotics are a large class of antibiotics that have a β -lactam ring in their molecular structure. They are the most widely used

antibiotics and include penicillin derivatives, cephalosporins, monobactams, carbapenems, and β -lactamase inhibitors.

Resistance to β -lactams involves several mechanisms, which are different in Gram-positive and Gram-negative bacteria. In Gram positive microbes, mutations, reduction of expression, or alterations in penicillin-binding proteins (PBPs) are the most important mechanisms, followed by β-lactamase production. Conversely, in Gram-negative microorganisms, the most prevalent mechanism of β-lactam resistance is the production of β-lactamases, followed by permeability alterations, extrusion by efflux pumps, and to a lesser extent PBP alterations.

PBP modifications

The PBPs involved in resistance and virulence are PBP2 (encoded by *mecA* in *Staphylococcus aureus*), PBP2b-PBPX (in *Streptococcus pneumoniae*), and PBP7-8 (in *A. baumannii*).

Previously reported data suggest that expression of homogeneous methicillin resistance in *S. aureus* influences the biofilm phenotype and attenuates virulence (it reduced protease production and significantly reduced virulence in a mouse model of device-related infection).

Clinical isolates of *S. aureus* can express biofilm phenotypes promoted by the major cell wall autolysin and the fibronectin (Fn)-binding proteins or the polysaccharide intercellular adhesin (PIA) and the polymeric *N*-acetylglucosamine (PNAG), which are synthesized and exported by proteins encoded by the *icaADBC* gene cluster.

Biofilm production in methicillin-susceptible *S. aureus* (MSSA) strains is associated with PIA/PNAG, whereas methicillin-resistant isolates express an Atl/FnBP-mediated biofilm phenotype (which produces a

proteinaceous biofilm) that suggests a relationship between biofilm production and susceptibility to β-lactam antibiotics.

Methicillin resistance reduces the virulence of health care-associated methicillin-resistant *S. aureus* (MRSA) by interfering with the *agr* quorum-sensing (QS) system in such a way that the ability of the bacteria to secrete cytolytic toxins is reduced. Methicillin resistance induces cell wall alterations that affect the Agr quorum-sensing system of the bacteria. This leads to reduced expression of the toxin and lowered virulence in a murine model of sepsis. This interesting finding may explain why some strains of hospital-acquired

MRSA show a reduced ability to spread in the community. It may also explain the recent increase in the incidence of community associated MRSA (CA-MRSA) strains, which typically express less penicillin-binding protein 2a (encoded by *mecA*) and thus maintain full virulence for success in the community setting.

Streptococcus pneumoniae was previously considered to be universally susceptible to penicillin. However, reports of isolates with decreased susceptibility to penicillin have increased worldwide in recent years. A virulent and penicillin-susceptible strain was transformed with *pbpX* and *pbp2b* from a penicillin resistant strain to assess the relationship between acquired resistance and virulence. After transformation, the virulence of these receptor strains was significantly reduced.

Beta lactamase production

Extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* strains have been suggested to possess higher pathogenic potential than non-producers. Although the adherence of ESBL- and non-ESBL-producing strains to epithelial cells did not differ significantly, the

proportion of strains able to invade epithelial cells was significantly higher among ESBL producers than among non-ESBL producers. Likewise, the proportion of ESBL producers coexpressing both fimbrial adhesins was significantly higher than that of non-ESBL producers. On acquisition of the ESBL SHV-12-encoding plasmids, two transconjugants started to produce type 3 fimbriae, although expression of type 1 fimbriae was not affected. Overall, the data demonstrated that an ESBL plasmid appeared to upregulate the expression of one or more genes, resulting in a higher invasion capacity. It remains unclear whether this effect resulted from direct SHV-type expression or expression of another plasmid-borne gene.

The impact of β-lactamases in relation to biological fitness costs in bacteria has been poorly studied. The interaction between resistance mechanisms and bacterial fitness will decide the fate of a specific bacterial strain once the selective pressure exerted by antibiotics disappears.

Permeability and porins

Porins are β-barrel membrane proteins that cross cell membranes and act as a pore through which molecules, such as nutrients, toxins, and antibiotics, can diffuse. Alterations, modification, and reduction in the expression of porins have all been associated with antimicrobial resistance to some extent. Porins have a clear role in virulence and resistance.

It is assumed that bacterial outer membrane proteins with a porin function not only control the entry of antimicrobials into bacterial cells but also control the virulence of the microorganism. The *A. baumannii* OmpA protein has recently been associated with resistance to cephalothin and cephaloridine in *A. baumannii*. Indeed, it has been reported that OmpA porin induces death of epithelial and dendritic human cells through mitochondrial targeting and is also involved in biofilm formation. Another

two porins, CarO and Omp33-36, have been associated with carbapenem resistance in this species. Other porins (such as OprD), initially associated with resistance to carbapenems. It has been demonstrated that attenuated virulence of a slow-growing pan-drug resistant *A. baumannii* strain associated with decreased expression of genes encoding the CarO and OprD-like porins.

Efflux pumps

Multidrug efflux pumps of bacterial pathogens are involved in intrinsic and acquired resistance to antimicrobial compounds, including those naturally present at mucosal surfaces; the pumps enable bacteria to grow on such surfaces and can thus be considered as being involved in colonization. Moreover, multidrug efflux pumps can discharge molecules involved in the quorum-sensing-regulated expression of virulence determinants.

Efflux pumps have also been shown to be important for detoxification of intracellular metabolites and are involved in bacterial virulence, as well as cell homeostasis and intercellular signal trafficking. Drug efflux can be driven by a proton gradient (such as resistance-nodulation-division [RND], small multidrug resistance [SMR], multidrug and toxic compound extrusion [MATE], and major facilitator superfamily [MFS] transporters) or by energy derived from ATP hydrolysis (ATP-binding cassette [ABC]).

The ability of bacterial pathogens to colonize, infect, and cause disease depends on their capacity to resist antibiotics, antimicrobial compounds produced by the host (such as bile acids, fatty acids, and other detergent-like molecules), and also components of the immune system (e.g., antimicrobial peptides). Clearly, such resistance may be mediated by active efflux systems belonging to the RND family of transporters, which

can extrude antimicrobial agents as well as the plethora of different compounds described above. Therefore, abrogation of efflux mechanisms undoubtedly affects both virulence and resistance to antibiotics in clinical practice, in terms of colonization and host and infection.

Resistance to Aminoglycosides and Effect on Virulence

Resistance to aminoglycosides is usually due to production of aminoglycoside-modifying enzymes, efflux pumps, Ribosomal methylases, and Ribosomal mutations.

Although chromosomal mutations that result in ribosomal alteration and resistance affect fitness, they will probably be selected in microorganisms only under high aminoglycosides pressure. This would explain why this mechanism is not often identified in the clinical setting. Thus, microorganisms will be selected by other antimicrobial resistance mechanisms with a lower fitness cost, such as aminoglycoside-modifying enzymes, which cannot yield any fitness cost in the absence of antibiotic pressure.

Resistance to Fluoroquinolones and Effect on Virulence

Several mechanisms of resistance to quinolones have been described: target modification (topoisomerase and DNA gyrase mutations), efflux pumps, Qnr (plasmid-mediated gene encoding quinolone resistance), porins, and quinolone-modifying enzymes.

The biological cost of quinolone resistance differs between bacterial species and depends on the level of resistance and the number of resistance mutations, and highly resistant mutants with multiple mutations show a significantly lower level of fitness than the wild type strains both in vivo and vitro. However, for low-level-resistant mutants with single mutations, the cost depends on the bacterial species.

It has been suggested that coselection of fluoroquinolone resistance and enhanced Type Three Secretion System-mediated virulence in *P. aeruginosa* clinical isolates harboring *exoU* (encoding a cytotoxin).

The coevolution of resistance to quinolones (mutations in *gyrA*, *gyrB*, *parC*, and *parE*) and increased virulence (expression of ExoU and ExoS), will favor the development of virulent genotypes, particularly in quinolone-rich environments.

How Does Increased Antimicrobial Resistance Affect Virulence?

There are no general solutions to the puzzle of evolution of antibiotic resistance and virulence, and the association between virulence and resistance in a specific pathogen will depend on the interactions between the multiple factors associated with bacteria and their environments.

The final effect (whether positive or negative) of the association between bacterial virulence and antimicrobial resistance depends mainly on four factors:

(i) The first factor is the bacterial species. Some microorganisms readily acquire antibiotic resistance mechanisms and evolve rapidly in response to antibiotic pressure (e.g., *P. aeruginosa* and *A. baumannii*). However, others remain fully susceptible to penicillin, although this has long been the treatment of choice for infections caused by the particular pathogen (e.g., *S. pyogenes*). Therefore, in the first type of bacteria, virulence will be more greatly influenced by the acquisition of resistance.

(ii) The second factor is specific virulence and resistance mechanisms. These are involved in both processes at the same time (e.g., the AcrAB-TolC efflux pump of *E. coli*, which expels fatty acids and bile salts and also antibiotics) or are involved indirectly (e.g., the

PhoP-regulated resistance to colistin in *P. aeruginosa*, which causes changes in the LPS and loss of affinity and is simultaneously involved in a decrease in virulence via lower biofilm production and lower cytotoxicity).

(iii) The third factor is the environment or ecological niche. This factor largely determines the development of the infection. Clear examples include the following: two-component systems, which regulate virulence and resistance at the same time and which depend on external stimuli; (1) the presence/absence of specific molecules (e.g., depletion of iron during the course of an infection in the host); (2) the antibiotic concentration, which depends on the anatomical site of an infection; and (3) a high NaCl concentration in the environment, which in *A. baumannii* may trigger a response that increases the resistance to several antibiotics through upregulation of efflux pumps and release of outer membrane proteins involved in virulence.

(iv) The fourth factor is the host (the immune system). Although in the strictest sense the host apparently does not affect the virulence of a pathogen, the host/pathogen interaction is key in the development of an infection and therefore in how the acquisition of resistance affects virulence.

MIMICRY AND BACTERIAL VIRULENCE

Lecture four

Dr. Harith J. Fahad
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The pressures of survival have engendered a fascinating spectrum of adaptations in organisms. Different organisms have evolved sophisticated methods to exploit the surrounding environment and each other. An important mechanism that frequently reoccurs in this process of adaptation is that of mimicry. Recent studies have begun to reveal that many bacterial pathogens mimic the function of host proteins to manipulate host physiology and cellular functions for the microbe's benefit.

Evolutionary mechanisms for mimicking the host

The ability to modulate cellular activities of the host at the molecular level through functional mimicry is a powerful tool for a bacterial pathogen. It allows the bacterium to be precise and limited in its effects, which can be useful in achieving its goals (for example, internalization into a host cell through limited disruption). However, obtaining virulence factors with such activity presents a daunting challenge. A pathogen may acquire such effector molecules by either obtaining "foreign" genes through horizontal transfer (in particular, host protein homologues), or through the process of convergent evolution.

Convergent evolution is the independent evolution of similar features in species of different lineages. Convergent evolution creates **analogous structures** that have similar form or function, but that were not present in the last common ancestor of those groups. The recurrent evolution of flight is a classic example of convergent evolution. Flying insects, birds, and bats have all evolved the capacity of flight independently. They have "converged" on this useful trait.

Mimicry through convergent evolution is perhaps the more intriguing of the two, as it involves taking `materials' (genes and the proteins that they encode) already available to the pathogen and then `sculpting' them to

perform a new function. Such a protein would usually have a distinct three dimensional architecture from that of the molecule it mimics, but would typically have evolved to imitate the chemical groups on the surface of its functional homologue.

For many of the functional mimics used by bacterial pathogens, the mimicry is indeed achieved through homologous enzymes that have been subverted for the benefit of the pathogen. These enzymes are often easily identifiable by sequence alignments as they contain highly conserved active sites or regulatory motifs.

These bacterial homologues of host proteins often differ from their host counterparts through alterations in substrate specificity, absence of regulatory control domains, and/or modulation of their intrinsic activity.

1- Host mimicry through convergent evolution

Many bacterial virulence factors with activities similar to host enzymes do not show any sequence similarity to eukaryotic proteins. As structural and functional similarities can occur even in the absence of sequence similarity. In some cases these bacterial mimics possess a structural architecture (the fold) that differs markedly from that of their host functional homologues. However, the molecular surfaces that interact with their targets, the true level at which natural selection ultimately sculpts, are seen as excellent mimics of proteins that operate normally in the cell. Two specific examples have been considered here where structural information has been a central element in understanding the function and evolution of a virulence factor.

Salmonella SptP and mimicry of signal transduction effectors

The enteric pathogen *Salmonella* delivers into the host cell two highly related bacterial proteins (SopE and SopE2) through a specialized

organelle termed the type III secretion system. These proteins function as guanine nucleotide exchange factors (GEFs) that activate Rac1 and Cdc42. Activation of these GTPases leads to profuse rearrangements of the actin cytoskeleton and subsequent bacterial internalization into intestinal epithelial cells.

Once safely inside the cell, *Salmonella* actively contributes to the restoration of the normal architecture of the host cell cytoskeleton by delivering another effector protein, SptP, thereby preventing any potential harm to its protected niche resulting from excessive Rho GTPase signalling. The amino-terminal half of SptP is a GTPase activating protein (GAP) for Rac1 and Cdc42.

In a precise matching of host cell function, SptP induces hydrolysis of GTP and shuts down the pathways controlled by the small GTPases that were activated by SopE and SopE2 (Figure 1).

The recently determined crystal structure of a SptP-Rac1 transition state complex reveals that the GAP domain of this effector, although possessing a new GAP fold, closely mimics host GAP enzymes. Notably, this mimicry occurs through a combination of structural elements, some of which mirror precisely the chemical groups and interactions from host proteins, whereas others use similar amino acids in new contexts.

Like host-cell GAPs, SptP extensively interacts with the regulatory Switch I and II regions of the GTPase, contacting similar residues-especially catalytic residues-but doing so in a very different manner from host enzymes.

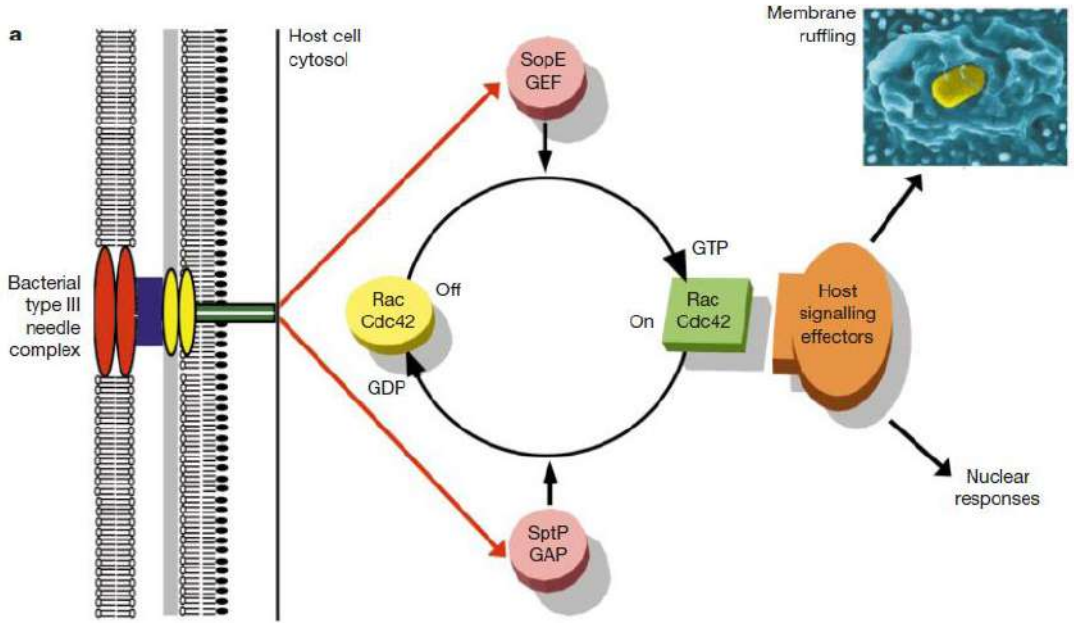

Figure 1: Host mimicry in the interaction of pathogenic bacteria with host cells. Interaction of *Salmonella* with host cells-striking a balance through mimicry.

Invasin and receptor substrate mimicry

Yersinia pseudotuberculosis uses the envelope protein invasin to bind host-cell β1 integrin surface receptors, thereby manipulating signal transduction pathways in the host and contributing to bacterial attachment and internalization. The potency of invasin is such that it will out-compete natural host substrates for β1 integrin binding (for example, fibronectin). No sequence similarity between invasin and host proteins can be detected, but the crystal structure of invasin reveals the effectiveness of convergent evolution in producing a virulence factor that mimics host activities. In this case, what is mimicked is the integrin-binding surface of fibronectin.

Numerous studies have revealed several important points regarding bacterial virulence:

A. Bacteria can have extremely subtle and sophisticated mechanisms for achieving their goals by means of fine-tuned and highly efficient biochemical processes;

B. Molecular mimicry of host proteins is a powerful tool exploited by many bacterial pathogens in the process of host manipulation.
C. Convergent evolution contributes significantly to the dynamics of the evolutionary process.

2- Molecular mimicry

Molecular mimicry is structural, functional or immunological similarities shared between macromolecules found on infectious pathogens and in host tissues. Which is considered one of the mechanisms that empower pathogenic organisms to avert or subvert the host's surveillance and defense mechanisms. The pressure to evolve molecular mimics may have arisen when the primordial free living organisms "chose" to parasitize other organisms. If a bacterial antigen (Ag) is very similar to normal host antigens, the immune responses to this Ag may be weak giving a degree of tolerance.

There are three types of molecular mimicry:

- *The first type* of molecular mimicry is identical amino acid sequences present in different protein molecules.
- *The second type* of molecular mimicry is due to structural similarities rather than amino acid sequence identities in the mimicking chemical structures.
- *The third type* of molecular mimicry is the recognition of completely dissimilar chemical structures on separate molecules by a single antibody.

Molecular mimicry and autoimmunity

Immunological mimicry between dissimilar epitopes on chemically different molecules has changed the concept that an antibody molecule must recognize only a single antigenic epitope.

Although molecular mimicry was once thought by immunologists to be a ‘phenomenon’ or ‘nonspecific immune reaction’, mimicry is now considered to be a part of the normal immune system and plays an important role in protection against pathogens.

Microbial infections may activate the immune system and lead to a loss of immune tolerance which could allow expansion of high avidity crossreactive B- or T-cell clones which through molecular mimicry may lead to autoimmune disease. Mimicry has been shown to lead to the induction of autoantibodies which alter signalling in cells.

In this case the antigenic determinants of the bacterium are so closely related chemically to host tissue components that the immunological cells cannot distinguish between the two and an immunological response cannot be raised. Some bacterial capsules are composed of polysaccharides (hyaluronic acid, sialic acid) so similar to host tissue polysaccharides that they are not immunogenic.

Molecular mimicry plays an important role in immune responses to infection and in autoimmune diseases. Infection may induce autoimmune responses which attack and destroy body tissues or organs. Normally, the body is tolerant to self-antigens which are present in individual tissues. In autoimmune disease, tolerance is abrogated to self-antigens, and tissues or organs are destroyed by the immune system.

Molecular mimicry of a self-antigen by an infectious pathogen, such as bacteria and viruses, may trigger autoimmune disease due to a

crossreactive immune response against the infection. Crossreactive antigen–antibody and T cell–antigen reactions are used to identify the mimicking macromolecules on the pathogen and in tissues or organs.

Examples of molecular mimicry

One of the classical examples of bacterial molecular mimics that elicit autoimmune reactions is the M protein of *Streptococcus pyogenes* that elicits autoantibodies that cross-react with heart myosin leading to heart damage.

In a form of molecular mimicry, gonococci make lipooligosaccharides (LOS) molecules that structurally resemble human cell membrane glycosphingolipids. The gonococcal LOS and the human glycosphingolipid of the same structural class react with the same monoclonal antibody, indicating the molecular mimicry. The presence on the gonococcal surface of the same surface structures as human cells helps gonococci evade immune recognition.

The terminal galactose of human glycosphingolipids is often conjugated with sialic acid. Sialic acid is a nine-carbon, 5-*N*-acetylated ketulosonic acid also called *N*-acetylneuraminic acid (NANA). Gonococci do not make sialic acid but do make a sialyltransferase that functions to take NANA from the human nucleotide sugar cytidine 5'-monophospho-*N*-acetylneuraminic acid (CMP-NANA) and place the NANA on the terminal galactose of a gonococcal acceptor LOS. This sialylation affects the pathogenesis of gonococcal infection. It makes the gonococci resistant to killing by the human antibody-complement system and interferes with gonococcal binding to receptors on phagocytic cells.

Prediction of molecular mimicry

Molecular mimicry of host proteins is a common strategy adopted by bacterial pathogens to interfere with and exploit host processes. Despite the availability of pathogen genomes, few studies have attempted to predict virulence-associated mimicry relationships directly from genomic sequences.

The proteomes of some pathogenic and nonpathogenic bacterial species were analyzed and screened for the top pathogen-specific or pathogen-enriched sequence similarities to human proteins. The screen identified many potential mimicry relationships including well-characterized examples among the top-scoring hits (e.g., RalF, internalin, yopH, and others), with about 1/3 of predicted relationships supported by existing literature.

Examination of homology to virulence factors, statistically enriched functions, and comparison with literature indicated that the detected mimics target key host structures (e.g., extracellular matrix, ECM) and pathways (e.g., cell adhesion, lipid metabolism, and immune signaling). The top-scoring and most widespread mimicry pattern detected among pathogens consisted of elevated sequence similarities to ECM proteins including collagens and leucine-rich repeat proteins.

Unexpectedly, analysis of the pathogen counterparts of these proteins revealed that they have evolved independently in different species of bacterial pathogens from separate repeat amplifications. Thus, our analysis provides evidence for two classes of mimics: complex proteins such as enzymes that have been acquired by eukaryote-to-pathogen horizontal transfer, and simpler repeat proteins that have independently evolved to mimic the host ECM.

Ultimately, computational detection of pathogen-specific and pathogen-enriched similarities to host proteins provides insights into potentially novel mimicry-mediated virulence mechanisms of pathogenic bacteria.

Antigenic Disguises

Some pathogens can hide their unique antigens from opsonizing antibodies or complement. Bacteria may be able to coat themselves with host proteins such as fibrin, fibronectin, or even immunolobulin molecules. In this way they are able to hide their own antigenic surface components from the immunological system.

Staphylococcus aureus produces cell-bound **coagulase** and **clumping factor** (clumping factor is a fibrinogen binding protein, which quietly different form coagulase) that cause fibrin to clot and to deposit on the cell surface. It is possible that this disguises the bacteria immunologically so that they are not readily identified as antigens and targets for an immunological response.

Protein A produced by *S. aureus*, and the analogous **Protein G** produced by *Streptococcus pyogenes*, bind the Fc portion of immunoglobulins, thus coating the bacteria with antibodies and canceling their opsonizing capacity by the disorientation.

The **fibronectin** coat of *Treponema pallidum* provides an immunological disguise for the spirochete.

E. coli K1, that causes meningitis in newborns, has a capsule composed predominantly of **sialic acid** providing an antigenic disguise, as does the hyaluronic acid capsule of *Streptococcus pyogenes*

ROLE OF FLAGELLA IN VIRULENCE

Lecture five

BY
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Introduction

Many Gram-positive and Gram-negative bacterial species and also Archaea as well as some eukaryotic cells have a flagellum. Flagellum is primarily a motility organelle that enables movement and chemotaxis. In addition to motility, flagella possess several other functions that differ between bacteria and during the bacterial life cycle: a flagellum can, for example, participate in biofilm formation, protein export, and adhesion.

The bacterial flagellum thus affects bacterial virulence in various ways, *i.e.*, by:

A. Promoting adherence and invasion.
B. Providing motility towards host targets.
C. Secreting virulence factors.
D. Promoting early biofilm formation and thus bacterial survival.
E. Triggering the adaptive and innate immune defense.

1. Flagella and adhesion

Typically, the adhesive structures are not expressed at the same time as the flagellum, so that movement and attachment occur one at a time. Thus, bacteria switch from motile to sessile lifestyle and *vice versa*, and these changes are triggered by different environmental conditions which regulate the expression of the *flhDC* flagellar master operon.

The regulation of flagellar expression occurs temporally at both the level of transcription and assembly. The flagellum has also been shown to function as an export apparatus that mediates extracellular secretion of non-flagellar virulence-associated effector proteins and biotechnologically important heterologous polypeptides.

There is a large body of published work for flagella-mediated adherence, yet there are very few examples of specific interactions where both flagella

and host determinants have been formally dissected. Instead, there is an emerging theme of nonspecific interactions, which has been challenging to investigate and likely relates to the biophysical properties of flagella:

Firstly, flagella organelles are long filaments that can reach up to 20 μm from the bacterial cell surface. It is therefore logical that flagella can be exploited as adhesive scaffolds and are involved in initial probing of surfaces as an early colonisation factor.

Secondly, its motor can spin flagella filaments at speeds in excess of 15,000 rpm, which not only increases the chances of the filament coming into contact with surfaces, but also ensures it does so with force. This is consistent with evidence that some flagella aren't adhesive, but are involved in cellular binding and invasion in processes distinct from providing niche proximity through propulsion. In the absence of specific protein receptors, observation of intercalation and penetration into plant and animal membrane lipid layers by flagella could also be explained by this phenomenon.

Thirdly, the flagellum filament is a polymeric structure, comprised of repeating epitopes of one or more flagellin types. Repeating epitopes are high avidity by definition: low affinity ionic interactions can be consolidated, amplified, and relevant if the binding substrate is also repetitive. With very few exceptions (innate immune receptors being the most notable), published examples of "specific" flagella binding interactions are with factors that are repetitive, such as polymeric proteins, proteoglycans, glycolipids, and phospholipids. Flagella therefore appear to be a tool with general properties that can be adapted to pathogenic colonisation of a diverse range of niches across plant and animal kingdoms.

2. Motility and Virulence

In many bacterial species, the flagellum is an acknowledged virulence factor, and non-flagellated strains have in several cases been observed to be

less virulent. The flagellum can act directly as an adhesin but can also affect virulence by other means.

Motility towards a host cell is a prerequisite for adhesion and invasion, and flagella can play an essential role in colonization by facilitating bacterial motility even if the flagella do not directly participate in the adhesion or invasion.

Early studies have shown that the single polar flagellum of *Vibrio cholerae* is crucial for its virulence: non-motile *V. cholerae* had reduced virulence in mice, and their adsorption to cross sections of mice intestine was decreased compared to motile *V. cholerae*. In *Salmonella enterica* serovar Enteritidis, studies with non-flagellated Δ*fliC* and flagellated but non-motile *motA* mutants have shown that functional flagella enhance the invasive capacity of the bacterium by enabling efficient motility.

Yet, the flagella filament *per se* is not required for adhesion and invasion. However, non-flagellated mutants of *S.* Enteritidis have been shown to cause significantly less of the typical invasion-associated membrane ruffling than the wild-type strain, indicating that flagella are involved in the early events of *S.* Enteritidis invasion.

Crude flagella from the opportunistic pathogen *Clostridium difficile* bind to cecal mucus of germ-free mice. In addition, non-flagellated *C. difficile* associate 10-fold lower with the cecal tissue than a flagellated strain, highlighting the role *in vivo* of flagella in adherence to mucus. In cystic fibrosis, *P. aeruginosa* colonizes the airway lumen at several micrometers from the surface. Noticeably, *P. aeruginosa* flagellin binds mucin Muc1, an abundant component of airway mucus. Furthermore, enteropathogenic *Escherichia coli* adhere to the intestinal mucosa or to tissue culture cells via flagellum-dependent mechanism. The hypervariable region of flagellin (D2-D3) is likely bearing the adhesin-like properties.

3. Flagellum Affects Virulence by Regulating Other Virulence Factors

Flagella can also contribute to virulence by regulating the expression of other virulence factors and the flagellum in some cases affects virulence in more than one manner.

Polar flagella are important virulence factors for *Helicobacter pylori*, because motility enables the bacteria to reach the gastric epithelium, adhere to it with several adhesins, and colonize the epithelium. Studies with a non-motile *fliD* mutant showed that FliD and thus a functional flagellum is required for the colonization of mice. It appeared that all flagellar mutants adhered to gastric cells, indicating that flagella do not play a direct role in adhesion of *H. pylori* and suggesting that in addition to regulating flagella, FlbA may regulate some *H. pylori* adhesins.

4. Flagellum as a secretion system for toxins

The machinery for flagellum biosynthesis is the paradigm of type III secretion system (TTSS). In pathogenic bacteria, TTSS serve as molecular syringes required for export and injection of virulence factors into the cytosol of host cells. Accordingly, pathogens hijack cytosolic pathways to colonize or kill the host cells. The flagellar TTSS can be an additional mechanism for export of virulence factors.

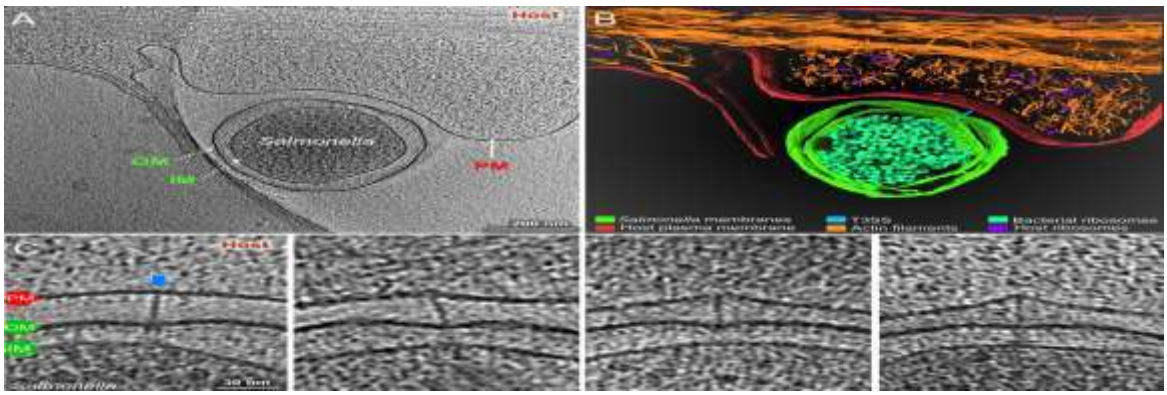

Figure 2: Type three secretion system mediated *Salmonella*-Host interactions.

5. Coordinated expression of virulence genes and genes involved in flagellum synthesis

The transcription of about 50 flagellar genes is hierarchically controlled by environmental conditions via the master regulator operon *flhDC*.

In *V. cholerae*, the ToxR regulatory system coordinates the transcription of motility genes and specific virulence genes in response to environmental conditions. In contrast, the BvgAS system of *Bordetella bronchiseptica* represses flagellum gene transcription while it activates the expression of virulence factors. In general, expression of flagella is likely switched off once mucosal pathogenic bacteria disseminate into deeper tissues.

The adhesive capacity of flagellin in combination with host ganglioside and toll-like receptor 5 (TLR5) appears as an efficient mechanism in host defense in the intestine as well as lungs but may also enable bacterial migration.

6. Flagella and biofilm

It is important to acknowledge that the notion of the motility-to-biofilm transition is largely theoretical. Biofilm formation takes place over prolonged periods of time during which the event of a cell or cells transiting between physiological states is difficult to capture. In addition, many bacteria are nonmotile, and some motile bacteria generate nonmotile subpopulations that may proceed directly to biofilm formation without further motility regulation. Despite these complications, the fact that some bacterial species can exist in two diametrically distinct modes of growth, either as motile individual cells or as nonmotile aggregates of a biofilm, prompts the inference that the bacteria must switch physiologies at some point. The inference of a transition is supported by mutations that enhance biofilm formation and reduce motility thereby revealing the reciprocal regulation of the two phenotypes. Thus, it has become clear that motility and biofilms are oppositely controlled but precisely

when, where, and how the transition takes place during biofilm formation is unclear.

The inhibition of motility is often thought to be an essential event in biofilm formation perhaps because motility is sometimes required early but inhibited late in mature biofilms. Furthermore, some mutants with enhanced motility are associated with decreased biofilm formation suggesting that the maintenance of motility may destabilize nascent multicellular aggregates. Empirically proving that motility inhibition is required for biofilm formation, however, is challenged by the fact that in many cases the mechanisms of motility inhibition are unknown, flagellar regulation is complex, and matrix biosynthesis gene expression is often coregulated with motility regulons. Even in instances where motility inhibition can be genetically separated from matrix synthesis, biofilm inhibition may appear transient due to rapidly selected suppressors that abolish motility through independent pathways. In fact, the arrival and dominance of nonmotile suppressors may be the best evidence that motility inhibition is important because they suggest that, under biofilm forming conditions, the selective pressure to inhibit motility is strong.

The concept of the motility-to-biofilm transition, regardless of biological importance, has proven powerful for the study of each individual phenotype. Understanding that the regulation of motility and biofilms is diametrically opposed has been critical in revealing the widespread importance of the secondary-messenger molecule c-di-GMP. Bacteria encode dozens of enzymes homologous to c-di-GMP synthases and hydrolases that control the cytoplasmic accumulation of c-di-GMP, which in turn promotes a biofilm-like state where motility is inhibited at multiple levels. The inputs that control c-di-GMP pools and the reason that mutation of various enzymes acting on a common pool gives rise to a spectrum of diverse phenotypes are unclear. Many output effectors of c-di-GMP remain to be discovered.

The relationship of motility to biofilms is complicated and likely invokes other regulatory systems like quorum sensing. Quorum sensing is a general term describing the process by which genes are regulated at high cell density in response to a secreted pheromone and is relevant as biofilms maintain cells at high density. In some systems, quorum sensing is necessary for EPS synthesis and biofilm formation whereas in other systems quorum sensing induces motility and biofilm dispersal. The differences in the way that quorum sensing regulates biofilms may be due to ecology. Inducing dispersion in *V. cholerae* may be advantageous in the water reservoir for colonizing new regions and in scavenging more nutrients.

INVASION

Lecture six

BY

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Mechanism of Bacterial Invasion:

Invasive bacteria actively induce their own uptake by phagocytosis in normally nonphagocytic cells and then either establish a protected niche within which they survive and replicate, or disseminate from cell to cell by means of an actin-based motility process. The mechanisms underlying bacterial entry, phagosome maturation, and dissemination reveal common strategies as well as unique tactics evolved by individual species to establish infection.

To establish and maintain a successful infection, microbial pathogens have evolved a variety of strategies to invade the host, avoid or resist the innate immune response, damage the cells, and multiply in specific and normally sterile regions. Based on their capacity to deal with these critical issues, bacteria can be grouped in different categories. Here we review the so-called invasive bacteria, i.e., bacteria that are able to induce their own phagocytosis into cells that are normally nonphagocytic. We focus on the tactics used by enteroinvasive bacteria to trigger their uptake by epithelial cells and discuss their intracellular life-styles. The mechanisms of entry and life-styles of other intracellular pathogens.

During phagocytosis by phagocytes, bacteria play a passive role. In contrast, during bacterial-induced phagocytosis, the bacterium is the key and active player in the complex interplay between the invading microbe and the host cell. Another important component is the cytoskeleton, whose plasticity is critical and optimally exploited. After internalization, some bacteria remain in a vacuole, in which they replicate. They prevent the normal maturation and trafficking of the phagosome and impair its normal bacteriolytic activities.

Other bacteria escape from the vacuole and replicate in the cytosol. In some cases, they also move and disseminate by means of an actin-based motility process.

How the cell senses the bacterial intruders and adjusts its transcription and translation programs to its new life with a parasite is an important issue. Apoptosis

and antiapoptosis, as well as cell cycle– and inflammation-related signaling pathways, are reprogrammed after infection to help the cell to survive the stress induced by the infection.

The success of an infection depends on the messages that the two players—the bacterium and the cell—send to each other. At each step of the infectious process, the bacterium exploits the host cell machinery to its own profit.

Entry Mechanisms

To enter nonphagocytic cells such as intestinal epithelial cells, some microbial pathogens express a surface protein able to bind eukaryotic surface receptors often involved in cellmatrix or cell-cell adherence. Expression of this protein leads to the formation of a vacuole that engulfs the bacterium through a “zippering” process in which relatively modest cytoskeletal rearrangements and membrane extensions occur in response to engagement of the receptor. The initial interactions between the bacterial protein and its receptor trigger a cascade of signals, including protein phosphorylations and/or recruitment of adaptors and effectors, and activation of cytoskeleton components that culminate in phagocytic cup closure and bacterial internalization. Other pathogens have devised mechanisms to bind a protein that can itself act as a bridge between the bacterium and a transmembrane receptor, which then mediates the entry process.

Finally, pathogens can also bypass the first step of adhesion and interact directly with the cellular machinery that regulates the actin cytoskeleton dynamics by injecting effectors through a dedicated secretory system. The effector molecules cause massive cytoskeletal changes that trigger the formation of a macropinocytic pocket, loosely bound to the bacterial body.

The Zipper Mechanism of Entry

Yersinia pseudotuberculosis and *Listeria monocytogenes* both harness transmembrane cell-adhesion proteins as receptors for entry into mammalian cells (Figs. 1A and 2A).

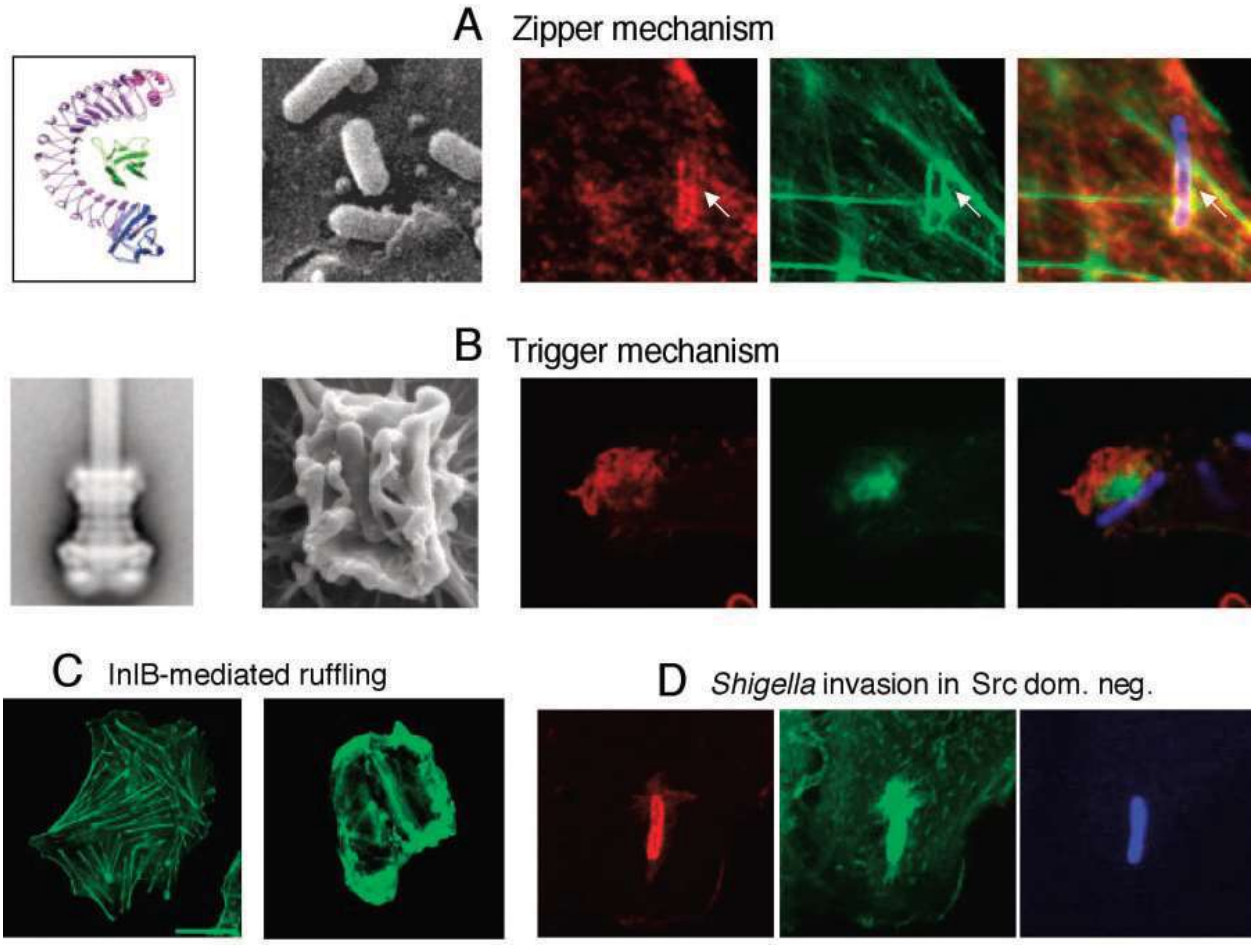

Figure 1: Zipper and Trigger mechanism

Entry can be divided into three successive steps: (i) Contact and adherence. This step is independent of the actin cytoskeleton and involves only the bacterial ligand and its receptor. It leads to receptor clustering. (ii) Phagocytic cup formation. This step is triggered by the transient signals occurring after formation of the first ligand-receptor complexes and propagating around the invading microbe. These signals

induce actin polymerization and membrane extension. (iii) Phagocytic cup closure and retraction, and actin depolymerization.

The *Yersinia* outer-membrane protein invasion binds to integrin receptors that have the _1 chain and are normally implicated in adherence of cells to the extracellular matrix. Invasin does not possess the RGD motif present in fibronectin, but both proteins interact with integrins by a structurally similar domain. Invasin has a higher affinity for integrins and can oligomerize, inducing integrin clustering and efficient downstream signaling.

The cytoplasmic tail of the _1 chain, hich normally interacts with the cytoskeleton in focal complexes of adhesion plaques, is critical for entry, but surprisingly, alterations of this domain that impair interaction with the cytoskeleton increase internalization. Thus, a lower affinity of the integrin for the cytoskeleton could allow higher mobility of the receptors in the membrane.

Activation of integrins leads to tyrosinephosphorylation events required for entry. The best-characterized protein, internalin (InlA), is a surface protein that is covalently anchored to the cell wall and belongs to a large family of leucine-rich repeat (LRR) proteins.

As for invasin, coating of latex beads with internalin promotes their entry, thus facilitating dissection of the specific pathway. Entry of *Listeria* into cells involves interaction between the LRR region of internalin and the first ectodomain of human E-cadherin, a transmembrane glycoprotein normally involved in hemophilic E-cadherin–E-cadherin interactions at adherens junctions of polarized epithelial cells. The LRR domain surrounds the first ectodomain of Ecadherin . This weak-affinity interaction cannot take place if proline-16 is changed into glutamic acid, as in murine E cadherin. Formation and maintenance of adherens junctions require the integrity of the E-cadherin cytoplasmic domain that binds catenins, which interact with the cell actin cytoskeleton. Similarly, entry of *Listeria* into cells requires the terminal 35

amino acids of E-cadherin. The latter binds to _-catenin, which recruits _-catenin, which in turn interacts with actin. Actin polymerization during internalinmediated entry is Rac dependent and mediated by Arp2/3, but how Arp2/3 is activated is unknown . Entry also requires an unconventional myosin, myosinVIIa, and its ligand vezatin . These two proteins probably play a role in the dynamics of the phagocytic cup. How the tension generated by the myosin motor is coupled to actin polymerization required for entry has not been established.

The second well-characterized *L. monocytogenes* invasion protein is InlB. This surface protein belongs to the LRR family of proteins and is only loosely attached by its C-terminal repeats to the bacterial surface, where it interacts with lipotechoic acids. Soluble InlB can reassociate with the bacterial surface of an InlB mutant and promote entry.

InlB interacts with three cellular ligands . The most relevant one is Met, a transmembrane receptor tyrosine kinase that upon interaction with its normal ligand, the hepatocyte growth factor (HGF), dimerizes and elicits phosphorylation on two critical residues that act as docking sites to recruit signaling and adaptor molecules. Met binding to the concave surface of the InlB LRRs also leads to its transient phosphorylation and to the recruitment and phosphorylation of the adaptor proteins Cbl, Gab1, and Shc, and activation of PI 3-kinase with the generation of PIP3 at the plasma membrane.

Autolysins Ami, Auto, and ActA contribute to *Listeria* adherence and entry. In addition, listeriolysin O (LLO), a pore-forming, cholesterol-dependent cytolysin involved mainly in escape from the internalization vacuole and that, like other toxins, interacts with lipid rafts, allows entry of extracellular calcium and stimulates entry.

Even in the absence of LLO, both internalin- and InlB-mediated entry are dependent on the presence of raft microdomains, suggesting that for entry, *Listeria*

take advantage of raft microdomains, which are known to be enriched in receptors and signaling molecules. Interestingly, cholesterol depletion does not affect the internalin- and InlB mediated pathways at the same step of the entry process .

The Trigger Mechanism of Entry

Both *Shigella* and *Salmonella* use this mechanism to enter the cell (Fig. 1B and Fig. 2B). Contact between bacteria and cells is mediated by the type III secretory system (TTSS) (Fig. 1). The TTSS allows direct activation of components of the cytoskeleton by delivery of dedicated bacterial effectors. In *Salmonella*, the TTSS is encoded by a chromosomal pathogenicity island (SPI-1) and in *Shigella* by a plasmid-located pathogenicity island (PAI).

These PAIs encode the structural components of the TTSS and some of their dedicated effectors. Two of these components (i.e., SipB/C in *Salmonella*, IpaB/C in *Shigella*) form a pore, or translocator, that delivers the effectors into the cell cytoplasm, creating a continuum between the bacterial and eukaryotic cytoplasms.

The interaction of bacteria with their epithelial cell target occurs in four successive stages:

1) **A pre-interaction stage**

   At 37°C, the effector molecules stored in the bacterial cytoplasm are associated with dedicated chaperones, whose major role is to avoid premature association of the effector molecules and their proteolytic Degradation. In exponentially growing bacteria, the TTSSs are properly assembled, but the secretion of effector proteins is repressed until the bacterium establishes contact with its cell target.

2) **An interaction stage**

   This stage encompasses complex events leading to the formation of a signaling platform. A recognition event is likely to take place at the tip of the TTSS, activating the secretory process via a retroactive signaling, possibly involving an

adenosine triphosphatase in the TTSS basal body. In *Shigella*, the high-affinity binding of IpaB to CD44—the hyaluronic acid receptor that is strongly expressed on the basolateral membrane of intestinal epithelial cells and on the surface of many other cell types, including cells of myeloid lineage—may be a key step in achieving transient adherence to the cell surface, activation of the secretory machinery, and insertion of the IpaB/C translocon into the eukaryotic cell membrane. Consistent with the association of CD44 with cholesterol and sphingolipid-rich membrane rafts, this step of the interaction is dependent on intact rafts. Cholesterol extraction disrupts binding to and entry into epithelial cells, and IpaB and CD44 segregate in these rafts. Similarly, in *Salmonella*, the protein components of the SipB/C translocon also segregate in rafts. The initial interaction may take place in these membrane subdomains because (i) the targeted receptor is enriched in rafts; (ii) the lipid composition of rafts is optimal for the formation of the pore and translocon, in a way similar to the cholesterol dependence of several hemolysins); and (iii) these domains are enriched in signaling molecules such as tyrosine kinases of the *src* family.

3) The formation of a macropinocytic pocket.

This stage involves localized but massive rearrangements of the cell surface, characterized by the formation of intricate filopodial and lamellipodial structures that appear similar in *Salmonella* and *Shigella*. Rearrangements of the actin cytoskeleton largely account for the formation of the entry focus. At the early stage of *Shigella* entry, VirA, a plasmid-encoded protein secreted through the TTSS, induces local destabilization of the microtubules that results in their depolymerization . The latter affects the early events of actin rearrangement through the deactivation of RhoA, leading to Rac1 activation and formation of Rac1-IRSp53-WAVE2 complex that recruits Arp2/3. IpaC in *Shigella* and SipC

in *Salmonella* (*37*) initiate actin nucleation through their C-terminal domain, which is exposed to the cytoplasm of the eukaryotic cell, via the IpaB/C or SipB/C pore. The mechanism of initial actin nucleation, however, remains uncertain. SipC can nucleate actin alone in vitro, but IpaC requires activation of Cdc42 and Rac 1 . Massive extension of the actin filaments that form entry foci seems to respond to different mechanisms in *Salmonella* and *Shigella*. In *Salmonella*, the translocated SopE proteins (SopE1 and SopE2) act as exchange factors for the Cdc42 and Rac-1 GTPases, thus massively boosting the initial nucleation event. Moreover, SopB/SigD, a TTSS-secreted phosphatidylinositol phosphatase, stimulates actin rearrangements and mediates bacterial entry, whereas SipA binds and stabilizes actin filaments Shigella has evolved a similar process of boosting cytoskeletal rearrangements, although through different molecular mechanisms. The C-terminal domain of IpaC is central to the activation of Cdc42 and Rac-1, which is quickly followed by activation of the tyrosine kinase c-src upon contact with IpaC, recruitment of cortactin to the membrane upon its c-src–mediated tyrosine phosphorylation, and further massive actin polymerization in the vicinity of the original actin cup via the Arp2/3 complex (Fig. 2C). This process is amplified by IpgD, a *Shigella* homolog of SopB/SigD. IpgD expresses a phospatidylinositol phosphatase activity that hydrolyzes PI(4,5)P2 into PI(5)P [phosphatidylinositol 5-phosphate], thus disconnecting the actin subcortical cytoskeleton from the membrane and favoring actin dynamics at the entry site. The Abl family of tyrosine kinases is also involved in *Shigella* entry through phosphorylation of the adaptor molecule Crk.

4) Actin depolymerization and closing of the macropinocytic pocket

This final stage is similar in *Shigella* and *Salmonella*, despite important differences between the effectors involved and the molecular mechanisms

exploited. In the case of *Salmonella*, SptP, a TTSS-secreted protein, has two activities: (i) a tyrosine-phosphatase activity that regulatesactivity of the mitogen-activated protein kinase (MAPK) induced by entry; and (ii) a GAP (GTPase-activating protein) activity on Cdc42 and Rac that antagonizes the activity of SopE, thus leading to shrinking of the entry focus by blocking further actin polymerization. It may seem strange that proteins of opposite functions are injected simultaneously into the target cell. Recent evidence indicates that, despite equivalent amounts delivered by the TTSS, SopE is rapidly degraded through a proteasomedependent pathway, whereas SptP is more stable. In the case of *Shigella*, IpaA, a TTSS-secreted protein, binds the N-terminal head domain of vinculin, a key protein in the formation of cell-adherence plaques, and induces actin depolymerization.

Stressosome and stress response

Lecture seven

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Stress response

An organism's survival from moment to moment depends, at least in part, on its ability to sense and respond to changes in its environment. Mechanisms for responding to environmental changes are universally present in living beings. For example, when mammals perceive a sudden environmental change as threatening, a rush of adrenaline precipitates the well-known “fight or flight” response. Such physiological stress responses in complex organisms require appropriately regulated interactions among numerous organ systems. But how do single-celled organisms respond to potentially lethal threats?

Pathogenic bacteria, unlike harmless commensals alternate between free living and host associated states. The physico-chemical parameters encountered by the bacteria in these two states are very different and exert different demands and stresses on the bacterial cell. Bacterial pathogens have evolved highly sophisticated mechanisms for sensing external conditions and respond by altering the pattern of gene expression with activation of a set of genes whose products assist in survival and turning off those the products of which are not necessary in a particular environment. These sensor-activator systems allow the bacteria to monitor environmental parameters which distinguish host from external environment and adjust gene expression accordingly, particularly by induction of virulence factors. The expression of virulence genes is controlled by regulatory systems in such a manner that the virulence factors are expressed at different stages of the infection process dictated by the changing micro-environment of the host as a consequence of the pathophysiology of infection. Accordingly, mutations in some of the regulatory systems attenuate virulence of several bacterial species.

The environmental control of regulatory mechanisms is mediated by complex processes both at the level of transcription and translation. Moreover, stress conditions like changes in the osmolarity of the growth medium,

anaerobiosis and temperature which pathogenic bacteria encounter upon entry into the host, can control gene expression by inducing changes in DNA topology which can provide an overlap between responses to different environmental stimuli.

Temperature stress

Induction of virulence genes

The first signal to an invading bacteria on entry into the host is an increase in temperature from that of the environment to the physiological temperature of the human body (37°C). In *salmonellae*, *shigellae*, *yersinae*, *Bordetella pertusis*, *Borrelia burgdorferi*, *Listeria monocytogens* and several other pathogenic organisms, the virulence gene cassettes are switched on at 37°C. In many of these pathogens, the virulence determinants are under the control of transcriptional activators which respond to fluctuations of growth temperature leading to an enhanced expression of virulence genes at 37°C.

Induction of heat shock genes

In addition to regulation of virulence genes in pathogenic organisms, temperature stress also induces the ubiquitous heat shock response involving the expression of a set of heat shock proteins (HSPs).

Temperature regulation of expression of virulence genes is distinct from the heat shock response in the character of the induction process. In heat shock response, the initial large increase in transcription of the heat shock genes is transient and is followed by the adaptation phase when the level of induction falls to a lower steady state value characteristic of the new elevated temperature. Induction of the virulence genes is more directly coupled to temperature and does not decrease unless temperature is lowered

Many of the HSPs are constitutively present in the cell even under no-stress situation and perform important house-keeping functions. The major HSPs are molecular chaperones that assist in correct folding and assembly of proteins and are involved in diverse cellular processes including: DNA replication, RNA transcription, flagella synthesis, UV mutagenesis and facilitates protein translocation across membrane barriers and possibly also secretion.

The fundamental functions of these HSPs are:

1. To prevent protein denaturation.
2. To reactivate partially denatured proteins.
3. Non repairable denatured proteins are degraded by another class of HSPs which represent either an ATP dependent protease or a catalytic or regulatory (ATPase) subunit of another protease.
4. HSPs participate in immune response to bacterial infections and development of autoimmune diseases.
5. Different classes of HSPs from different bacteria can directly induce cytokine expressions and secretion in macrophage.

3. Oxygen stress

The expression of adherence and invasion factors of several pathogenic bacteria is regulated by oxygen concentration. High oxygen usually represses whereas low oxygen induces invasiveness. During switch from aerobic to anaerobic growth condition, a set of genes are induced and some genes are repressed. In *E. coli* two regulatory mechanisms have been identified which control the expression of these genes. One regulatory network is the Fnr (fumerate-nitrate reductase)-dependent control in response to anaerobiosis. Fnr activates the transcription of several respiratory genes such as fumarate reductase (*frd*), dimethyl sulphoxide-triethylamine-N-oxide reductase (*dms*)

and nitrate reductase (*nar*) and represses the expression of cytochrome d (*cyd*) operon. The *frd* and *nar* gene products are required for the reduction of fumarate and nitrate so that they can serve as alternative electron acceptors for oxidative phosphorylation even in the absence of oxygen**.**

Osmotic stress

For a pathogenic bacterium which passes from environmental waters to the human body for infection, osmolarity is an important criterion to distinguish between the external and host associated environments. Osmolarity of an aqueous environment is thought to be no greater than that equivalent to 0·06 M NaCl while in the intestinal lumen the osmolarity is much higher (equivalent to 0.3 M NaCl) and in the blood stream the bacteria encounter an osmolarity equivalent to about 0.15 M NaCl. Thus, an increase in osmolarity is associated with expression of virulence factors in many pathogenic organisms. In *S. flexineri,* expression of the plasmid located *vir* genes which are necessary for invasion of epithelial cells is markedly enhanced under conditions of high osmolarity (0·15 M NaCl)..

Pseudomonas aeruginosa, which infects cystic fibrosis patients, synthesizes alginate, a capsular polysaccharide, necessary for maintenance of virulence of the organism. High osmolarity in the lungs of cystic fibrosis patients is one of the signals that contribute to the increased transcription of *alg*D gene encoding GDP mannose dehydrogenase, a primary controlling factor in the alginate biosynthesis.

The Role of Sigma Factors in Transcription

In bacteria, alterations in gene expression are often controlled at the transcriptional level through changes in associations between the catalytic core of RNA polymerase and the different sigma factors present in a bacterial cell. RNA polymerase is the enzyme responsible for recognizing appropriate

genes under specific environmental conditions, and for creating the mRNA transcripts that can be translated into new proteins. Sigma factors are dissociable subunits of prokaryotic RNA polymerase. When a sigma factor associates with a core RNA polymerase to form RNA polymerase holoenzyme, it directs the holoenzyme to recognize conserved DNA motifs called promoter sites (or regions) that precede gene sequences. Sigma factors also contribute to DNA strand separation, which is a critical step in transcription initiation. The sigma subunit dissociates from the RNA polymerase core enzyme shortly after transcription begins, thus becoming available for reassociation. Associations between different alternative sigma factors and core RNA polymerase essentially reprogram the ability of the RNA polymerase holoenzyme to recognize different promoter sequences and express entirely new sets of target genes. As the set of genes controlled by a single sigma factor (also known as the regulon) can number in the hundreds, sigma factors provide effective mechanisms for simultaneously regulating large numbers of prokaryotic genes.

How do single-celled organisms respond to potentially lethal threats?

Sigma factors are classified into two structurally unrelated families: σ^{54} and σ^{70} families.

Subunits comprising the σ^{54} family are often commonly referred to as σ^{N}. σ^{N} has been identified in multiple diverse species, including *Legionella pneumophila*, *Pseudomonas* spp., *Enterococcus faecalis*, *Campylobacter jejuni*, and *L. monocytogenes*. In addition to regulating nitrogen metabolism in a number of organisms, σ^{N}-dependent genes also contribute to a diverse array of metabolic processes.

σ^{70} family members, which is larger and more diverse than the σ^{54} family, that contribute to bacterial stress responses (e.g., σ^{S} (RpoS) and σ^{B} (SigB)

have been identified as general stress responsive alternative sigma factors in Gram-negative and in Gram-positive bacteria, respectively) are of particular interest as mounting evidence suggests that in bacterial pathogens, these regulatory proteins serve as links between bacterial abilities to respond to changes imposed by the host environment and, subsequently, to cause disease.

The σ^{70} family is divided into four groups based on conservation of their primary sequences and structures:

1. The Group I sigma proteins are the primary sigma factors (e.g., *Bacillus subtilis* σ^{A}) and are also referred to as "housekeeping" sigma factors, as they direct transcription of genes important for bacterial growth and metabolism.
2. Group II sigma factor (σ^{S} was identified in both *E. coli* and in *S. typhimurium*) activates:
    a) Expression of numerous genes required to maintain cell viability as the cell leaves exponential growth conditions and moves into stationary phase.
    b) Contributes to expression of virulence-associated genes.
3. Group III sigma factor (σ^{B}) was initially identified and characterized in *B. subtilis*, but has also been identified in *L. monocytogenes*, *Staphylococcus aureus*, *B. anthracis*, and *B. licheniformis*. The *B. subtilis* σ^{B}-dependent general stress regulon is large: over 200 genes are expressed following bacterial exposure to heat, acid, ethanol, salt stress, entry into stationary phase, or starvation for glucose, oxygen, or phosphate. In *L. monocytogenes*, σ^{B} contributes to expression of internalin A and internalin B, two bacterial surface-associated proteins important for host-cell invasion.
4. Group IV sigma factors, the extracytoplasmic function (ECF) are conserved across both Gram-positive and Gram-negative species, and

comprise a large, phylogenetically distinct subfamily within the σ^{70} family. Members of the ECF subfamily are distinct from the rest of the σ^{70} family in that they regulate a wide range of functions involved in sensing of the extracellular environment and reacting to conditions in the membrane, periplasm, or extracellular environment. **σ^{E}, an ECF sigma** responds to accumulation of specific unfolded proteins in the periplasm and it may represent an important switch mechanism that facilitates bacterial transition from a free-living organism to a host-invading pathogen.

Stressosome

A "co-ordination centre" that directs a bacteria's defence systems to fight off external threats and instead of a single receptor system; a network of sensors connected to a signalling hub, which is known as "stressosome".

The stressosome initiates a "global instantaneous response" to the stress, which results in the creation of an army of new proteins to counterattack the threat or stress. Rather than being on or off, the stressosome is able to modulate the signal in proportion to the stressful situation.

Although the stressosome is the command centre, the system relies heavily on a protein, named RsbT, to communicate that danger. When the stressosome receives a warning signal that a dangerous situation is present, the RsbT protein breaks away from the molecule. Thereafter, transfers information to the bacteria's genetic apparatus that causes new proteins to be produced. These are then used to help the cell to adapt to the new environment, or restore the environment to the norm.

For example in an environment that became too salty, the bacteria would produce a set of proteins that would eliminate the salt and restore it to the right level.

Researchers are now looking at the role of the RsbT protein and how it adheres and falls away from the stressosome hub.

In growing cells, σ^B is present but sequestered in an inactive state by its antagonist, RsbW. Stress conditions cause RsbW to switch its partner and to bind the anti-anti-sigma factor RsbV resulting in the release of functionally active σ^B. In growing cells, RsbV is maintained in its phosphorylated state which is not bound by RsbW. Only upon stress, when phospho-RsbV is dephosphorylated, can RsbV become an interaction partner for RsbW (fig. 1). Indeed, the affinity of RsbW for non-phosphorylated RsbV is about 8-fold higher than for σ^B, whereas no interaction of RsbW with phosphorylated RsbV can be detected. Thus, the control of the RsbV phosphorylation state is crucial for σ^B activity and, consequently, for the expression of the stress genes. RsbV is phosphorylated in growing cells by RsbW, which has an RsbV-specific protein kinase activity in addition to its function as an antisigma factor. Dephosphorylation of phospho-RsbV is performed by two RsbVP-specific protein phosphatases, RsbP and RsbU. RsbP dephosphorylates RsbV-P under conditions of energy limitation whereas RsbU is active during environmental stress (fig. 1). The phosphatase activity of RsbU under stress conditions is triggered by its interaction with another regulatory protein, RsbT. RsbT is sequestered in the absence of stress in a protein complex comprising at least RsbR and RsbS, and also paralogues of RsbR . This > 1-MDa complex has a ring-like structure that has been called the stressosome (fig. 1). In vitro and in vivo data imply that both RsbR and RsbS are required in order to act as antagonists in partner switching. In vitro data suggest that RsbT dissociates from the stressosome under stress conditions upon RsbT-mediated phosphorylation of RsbR and RsbS. This model is further supported by the finding that in vivo phosphorylation of RsbR and RsbS correlates with stress transmission. The release of RsbT from

the stressosome allows its interaction with and concomitant activation of the RsbU phosphatase that dephosphorylates RsbV-P and forces the partner switching of RsbW and subsequent release of σ^B leading to the transcription of σ^B -dependent genes (fig. 1). In order to reset the signaling system, the phosphorylation of RsbR and RsbS can be reversed by the action of the RsbX protein phosphatase. However, the control of RsbX activity remains to be elucidated. Recently, the *B. subtilis* RsbR protein was re-designated RsbRA.

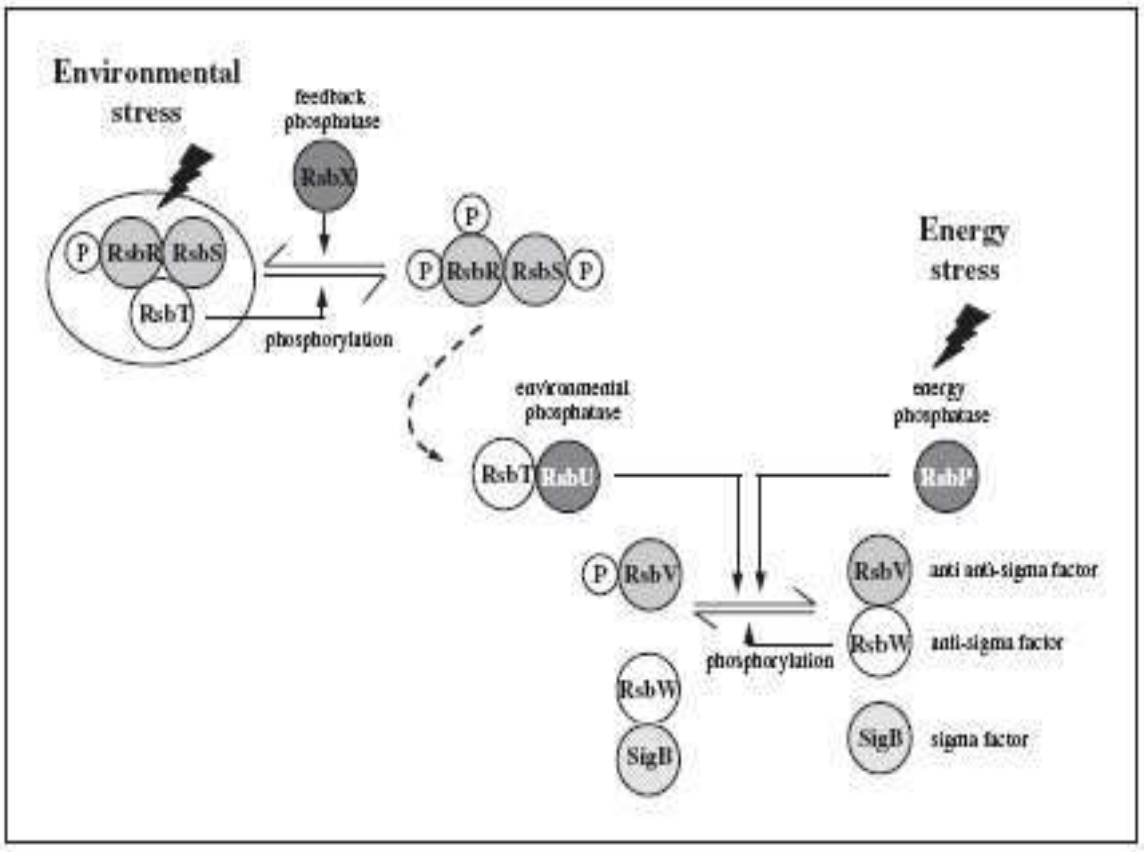

Figure 1: The signalling pathways controlling the activity of σ^B activity in *B. subtilis* .

MECHANISM OF CELL DEATH IN RESPONSE TO BACTERIAL INFECTION

Lecture eight

BY

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Mechanism of Host Cell Death

Host cell death has been a very significant focus of biological research for years. A broad array of investigations and studies is concerned with this persuasive fact. Death of cells can occur naturally as an obvious phenomenon in cell cycle for the development of tissues and organs systematically to prevent the body from impairment or functional disorder. However, this cell death can also be induced by pathogens like bacteria, viruses etc. Host cells in response to bacterial infections; die as a result of host-microbe interaction (Figure 1).

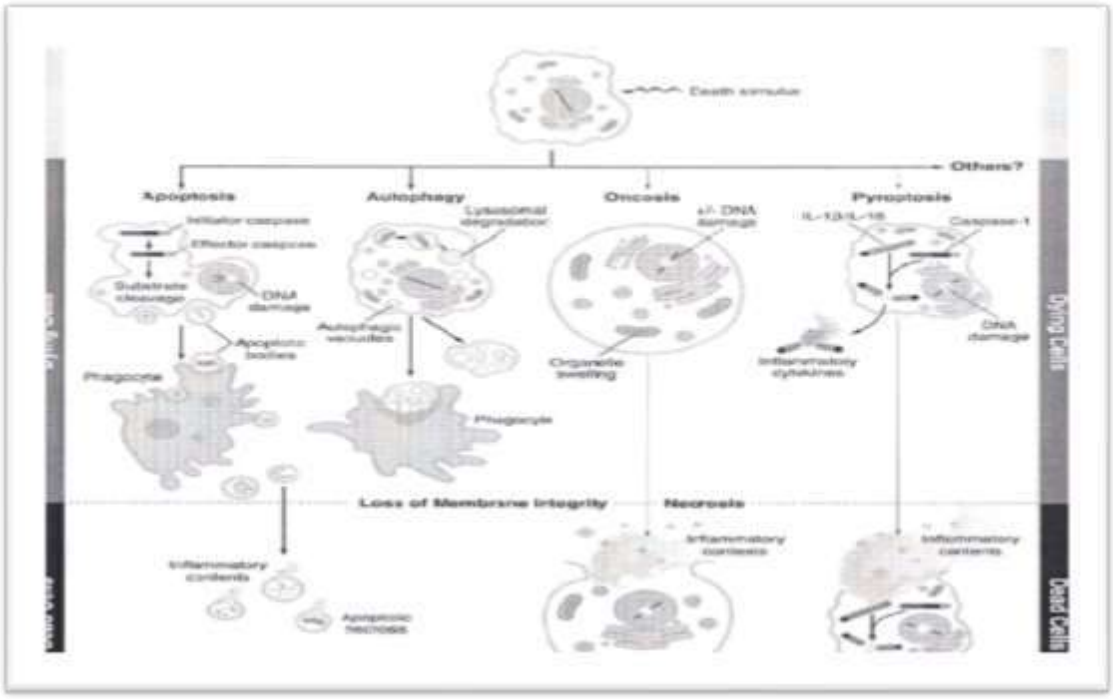

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Figure 1: Different pathways showing host-pathogen interaction resulting in characteristic disintegration of the host cell membrane

Cells before undergoing degeneration receive specific chemical signals at particular stages of infection, and these signals trigger the activation of appropriate molecules, which eventually guide the particular cell to death. Bacteria result in damage of host cells by releasing a wide range of virulence factors and suppressing

cell growth factors, and thereby interfering with cell growth regulation. Different types of cell death have been demonstrated in past and recent studies. Of these, apoptosis and necrosis are widely elucidated with regard to bacterial pathogenesis. Nonetheless, some other pathways of cell demise have also drawn attention to a large extent.

The key objectives of this study are to understand the reasons why a cell is subjected to death and the appropriate machinery implicated in it, and to explore the magnitude of benefits gained from this occurrence by both the host and Pathogens.

Comparative Study of Different Types of Cell Death

Apoptosis and necrosis

Although both apoptosis and necrosis are forms of cell death, they differ from each other considerably. Apoptosis is considered as an extremely harmonized route of cell death, and is requisite for the growth and homeostasis of multicellular organisms. Furthermore, unlike necrosis, apoptosis does not induce inflammatory response during the process of removal of discrete cells. Vesicles are produced in cells that apoptose without the release of any cellular content. However, death by necrosis is characterized by cell enlargement and lysis, and is able to cause inflammatory responses as a consequence. It is thought that apoptosis is an active, programmed process of independent cellular disintegration, whereas necrosis is a passive, accidental cell death accompanied by uncontrolled discharge of inflammatory cellular contents that takes place due to environmental disturbance. Morphologically, apoptosis is regarded as a general pathway of shielded cell deletion corresponding to mitosis and cytokinesis that upholds stable populations within tissues. Hence, it is now considered that living cells are genetically programmed to consist of metabolic components leading to cellular demise upon activation. On the contrary, studies reveal that cell necrosis has different mechanisms and outcomes. During necrosis cells first swell, and then the plasma membrane breaks down and

cells rapidly undergo lysis. But, during apoptosis cellular shrinkage and corresponding DNA condensation occur initially, and thereafter these cells and nuclei disintegrate into well-enclosed apoptotic bodies. If the apoptotic bodies are not phagocytized, there could be a loss of integrity in apoptotic bodies leading to secondary or apoptotic necrosis. It implies that further degradation of these bodies may possibly take place after the apoptotic course. Table 1 represents certain key characteristics of apoptosis and necrosis.

Oncosis

It is also known as Early Primary Necrosis. The term oncosis Reckling-Hausen exactly to mean cell death with swelling. Cells are directed to necrosis with karyolysis (dissolution of nucleus) by oncosis, while karyorhexis (fragmentation of nucleus) and cell shrinkage are the key characteristics of apoptotic necrosis. In other words, oncotic cell death is measured by cytoplasmic bulging, mechanical rupture of the plasma membrane and expansion of cytoplasmic organelles like mitochondria, endoplasmic reticulum and Golgi apparatus, as well as moderate chromatin condensation. Thus, oncosis fairly resembles necrosis in terms of the cellular destiny. Furthermore, bacteria-induced oncosis has been discerned in many experiments. *Pseudomonas aeruginosa* infection brings about oncosis in infected macrophages and neutrophils. In these cells, swelling, rapid plasma membrane collapse, and inflated nuclei without inter-nucleosomal DNA disintegration are evident.

Autophagy

Autophagy is another mode of cellular disintegration, which is thought to be of great importance in cell life cycle. Autophagy is known as a non-specific degradative pathway that is engaged in the transportation of bulk cytoplasmic components to the vacuole.

Perhaps, autophagy is another nature of programmed cell death, which is regarded as Type II PCD (Programmed Cell Death) and is allied with Type I PCD, apoptosis. Apoptosis is followed by the exclusion and degradation of dying cells by phagocytosis, which is a sign of autophagy. The morphological features of autophagy comprise vacuolization, deterioration of cytoplasmic contents, and minor chromatin condensation.

Pyroptosis

Research findings propose that pyroptosis, a new form of death, occurs in host cells that exhibit apoptotic circumstances. It is generally characterized by the pro-inflammatory nature of cell death process and is believed to have medical significance in infections induced by certain bacteria like *Salmonella typhimurium*. *Salmonella*-induced cell death has been regarded as necrotic in nature based on the cytotoxicity generated by this bacterium. Pyroptosis is now contemplated as a pro-inflammatory programmed cell death because of the requirement of caspase-1 activation and the pro-inflammatory route in this mechanism. Therefore, it should also be noted that pyroptosis is a non-apoptotic programmed cell death because of its exceptional dependence on caspase-1. Caspase-1 is not concerned in orthodox apoptotic cell death pathway and particularly caspase-1 deficient cells react normally to most apoptotic signals. The development of active inflammatory cytokines, IL-1β and IL-18 (interleukins) is a notable role of caspase-1. Pyroptosis is believed to have further relevance in a diversity of biological approaches due to the observation of caspase-1 activation or dependence during cell death in the immune, central nervous and cardiovascular systems.

Factors Triggering Cell Death

Death receptors

Death receptors are transmembrane proteins that belong to tumor necrosis factor (TNF) receptor (TNFR) super family, and localized to the cytoplasmic

membrane. The extracellular domains of these receptors are engaged in ligand binding and their “death domains” (DDs) in cytoplasmic tails facilitate apoptotic machinery. Generally, death receptor function can be demonstrated by Fas (CD9S/APO-1) as it is a member of the TNFR family. Polymerization of Fas leads to onfiguration of the death inducing signal complex (DISC). During this mechanism, Fas- associated death domain (FADD) protein, an adaptor molecule, is engaged through death domains (DDs). FADD also contains two death effector domains (DEDs) that adhere to caspase-8 or its enzymatically inactive homologue, the Fas inhibitor FLICE (FADDlike interleukin-1B converting enzyme) inhibitory protein (FLIP). Thus, it can be suggested that various cellular receptors can mediate signaling for programmed cell death.

Mitochondria

Mitochondria have been recognized as vital organelles in cell death. The cytosolic assembly of apoptosome (apoptosis inducing caspase activation complex involving Apafl and caspase-9) has been observed in mammals upon discharge of mitochondrial cytochrome c. Likewise, mitochondrial swelling, outer membrane rupture and the release of apoptotic mediators can be attributed to mitochondrial permeability transition (mPT). For this reason, mitochondria play an important role in programmed cell death.

Caspases

As discussed earlier, in certain types of cell death, such as apoptosis and pyroptosis, caspase molecules are largely influential. They constitute a group of cysteine proteases that cleave at caspase-specific sites.

Apoptosis seems to be caspase-mediated cell death because an array of caspases (caspase-2,-3,-6,-7,-8,-9, and -10) is recruited in the route of apoptotic cell death.

Mechanisms of Host Cell Death Induced by Bacterial Infections

The vigorous interaction between eukaryotes (host cells) and prokaryotes (bacteria) is a very complex ideology of medical science in view of the involvement of diverse effectors, mediators and toxins leading to an extensive array of pathways. Therefore, it is sort of challenge to localize all facets of this very obvious natural phenomenon.

However, in this review, feasible efforts will be made to explain assorted modes of cell fatality while encountering bacterial pathogens. In order to comprehend how cellular breakdown or malfunction is triggered by bacteria, possible effects of hostile factors of these pathogens need to be detailed. Many pathogenic bacteria are equipped with a wide range of virulence determinants, which interact with vital components of the host leading to cell death. These determinants may also barricade the regulation of transcription factors, which are recruited in monitoring cell survival. Diverse bacterial exotoxins have the ability to bring about direct lysis of cells and ultimately help with microbial spread through tissues by causing momentous damage to the extracellular matrix or the plasma membrane of eukaryotic cells. Perhaps, these toxins result in this cellular injury by dint of enzymatic hydrolysis or pore development. In view of proven information, bacterial hyaluronidases, collagenases, and phospholipases are capable of decaying cellular membrane or matrices. Cell death pathways with respect to the impact of different toxins can be typified by certain bacteria for a reasonable perception.

Pore Formers vs Host Cells

A wide range of bacteria produces pore-forming toxins that interfere with the cell cycle regulation. For instance, these toxins upset the selective mobilization of ions across the plasma membrane by introducing a transmembrane pore. Both gram-positive and gram negative bacteria produce pore-forming toxins, such as the RTX

(repeats in toxin) toxins produced by certain gram-negative bacteria, streptolysin O by *Streptococcus pyogenes*, and the *Staphylococcus aureus* α-toxin.

Staphylococcus aureus

Staphylococcus aureus is the causative agent of a variety of diseases, such as skin lesions, food poisoning, toxic shock syndrome, endocarditis, and osteomyelitis. Alphatoxin has been recognized as the most important haemolysin of this bacterium that prthrough the classical apoptotic pathway. Alpha toxin forms pores in a number of eukaryotic cell membranes and activates programmed cell death in T-lymphocytes. At low doses, the toxin connects to specific cell surface receptors. Then it produces miniature pores resulting in the ease of the release of monovalent ions. Finally, these events facilitate DNA fragmentation and cell death. More importantly, alpha-toxin non-specifically absorbs to the lipid bilayer at high doses inducing the formation of larger pores that happen to be Ca2+ permissive resulting in substantial necrosis without DNA fragmentation. Other studies express that α-toxin undergoes a set of precise steps during access to host cells in order to damage cell membranes. It selectively carries out three sequential events. First, toxin protomers possibly bind to target membranes by high-affinity receptors or through non-specific assimilation to phosphotidylcholine or cholesterol like substances on

the lipid bilayer. Then membrane-bound protomers, oligomerize to generate a heptamer complex capable of forming a pore. In the end, the heptamer goes through a succession of conformational changes that trigger the formation of the stem domain of the toxin, which is then inserted into the membrane. The o-toxin pore facilitates the influx and efflux of small molecules and ions leading to characteristic swelling and death of nucleated cells and osmotic lysis of erythrocytes.

Escherichia coli

Another RTX toxin, which is produced by *Escherichia coli*, is called alpha-haemolysin (HlyA) infection with this pathogen accounts for diseases like severe

bloody diarrhoea, abdominal cramps, and haemolytic uremic syndrome particularly in children. HlyA mediates cell death via LFA-1 (lymphocyte function-associated antigen 1) in human immune cells. Attempts have been made to understand the possible interaction between *E. coli* and host cells. As an outcome, host cells have shown certain morphological changes that confirm the role of alpha-haemolysin in the cell death pathway. For instance, cytoskeleton rearrangement is found in erythrocytes exposed to *E. coli* HlyA that eventually brings about the formation of teardrop-shaped protuberance from the surface. The production of cytokine is also disturbed by alphahaemolysin . This pore-forming toxin is primarily virulent in its acylated form. The species of the acylated form inserts as a monomer into the plasma membrane bilayer of target mammalian cells in order to create a transmembrane pore for the permeability of cations over anions.

Infection with Protein Synthesis Inhibitors

An assortment of bacterial species is concerned with this group. These pathogens are responsible for the type of host cell death that is triggered by protein synthesis inhibition, and are capable of doing so by the secretion of specific bacterial proteins (toxins), which have intrinsic enzymatic activity. These proteins consist of an A-B conformation with the B subunit being able to mediate ligation with eukaryotic cells and the enzymatically operational A fragment translocated across the cell membrane to its cytosolic destination.

Some of the examples of these proteins are diphtheria toxin (Dtx) secreted from *Corynebacterium diphtheriae*, Pseudomonas exotoxin A, and the Shiga and Shiga-like toxins .

Corynebacterium diphtheriae

This gram-posovokes cell death***Corynebacterium diphtheria*** This gram-positive rod is the predominant cause of diphtheria in humans and well identified as

an extracellular pathogen. Infection takes upon interaction with host cells across plasma membrane.

Diphtheria toxin (DT) also described as A-B toxin binds to the DT receptor on the host cell surface through its B fragment. This binding allows internalization of the toxin into acidic endocytic vesicles leading to the liberation of catalytic DT-A into the cytoplasm, where this subunit implements its cytotoxicity by ADP-ribosylating elongation factor 2 (EF-2). This mechanism, eventually, hinders protein synthesis in infected cells, thereby killing them. Experimental evidences further strengthen the notion of the cytotoxic effect of diphtheria toxin on host cells. In a previous study, transgenic mice expressing human pro-HB-EGF [heparin-binding EGF (epidermal growth factor)-like growth factor, recognized as DT receptor was infected with diphtheria toxin by intramuscular injection. This experiment brought about complete ablation of transgene-representing cardiomyocytes in mice and subsequent heart failure resulting from the autophagic cell death pathway. However, it is also considerable that Dtx cannot plot the destiny of infected host cells by means of solely protein synthesis inhibition because certain observations have reported the absence of cellular apoptosis despite effective obstruction of this synthesis in cells.

A host nuclear transport factor, cellular apoptosis susceptibility (CAS) protein, is suspected to be engaged by Dtx-mediated apoptosis in order to enhance the possibility of death of target cells. To epitomize, on the basis of the explained protein synthesis inhibition route, the true mechanism of *Corynebacterium* (Dtx)-infected host cell death still remains vague in studies.

Shigella dysenteriae

This pathogenic species is one of the major causes of dysenteric syndromes in humans with typical kidney and central nervous system manifestations. Toxins generated by this bacterium are Shiga and Shiga-like toxins (Slt) and verotoxin that have been experimentally proven to be efficient in Shigella-caused route of cell

death by launching inhibition of protein synthesis. A subunit of Shiga toxin has capacity to cleave a single adenine residue from the 28S rRNA component of eukaryotic ribosomes by its N-glycosidase activity. Thus, ribosome function is disturbed ending in disruption of protein synthesis.

Past studies failed to detect the important accountability of Shiga toxin secreted from *Shigella dysenteriae* 1 in this mechanism. Research in the last decade years, conversely, has developed knowledge about the apparent effect of this toxin on rectal mucosa infected with *S. dysenteriae* 1 and improved our understanding of its possible mechanism leading to cell death. Interestingly enough, both apoptosis and necrosis were observed in infected rectal tissues. A marked upregulation of Fas colocalized to cells was found to be associated with apoptosis, whereas necrosis mediated by perforin, a protein of natural killer cells, was mostly discernible in the surface epithelium. Also, *in vitro* studies with peripheral blood mononuclear cells have established that Shiga toxin induces a rapid Fas-assisted cell death with a decline in Bcl-2 expression. These consequences reveal that *S. dysenteriae* 1 adopts such a mechanistic strategy upon contact with host cells that results in initial Fas-associated apoptosis followed by elimination of *Shigella* antigen positive cells by perforin-activated cytotoxicity at a later phase.

Execution by Type-III Protein Secretion Pathway

Type-III protein secretion is a novel pathway, which is now thought to be broadly functional in the interaction of pathogenic bacteria with host cells. It has been observed in diverse gram-negative bacteria, such as *Salmonella, Shigella, Yersinia,* and enteropathogenic *Escherichia coli* (EPEC) that are highly pathogenic to animals. This protein secretion system is also known as contact-dependent system. Certain distinctive the export of the target proteins through both the inner and surface membranes. Most importantly, this protein secretion system can be completely efficient provided that extracellular signals are activated. Equally, Type-III secreted

proteins are aimed at changing host cell signal transduction pathways upon interaction. Mechanism of this critical pathway can be sensibly explained with respect to an assortment of certain pathogenic bacterial species causing infection to host cells.

Salmonella typhimurium

This species of *Salmonella* genus is basically responsible for gastroenteritis. Virulence genes of this species are particularly positioned in *Salmonella* pathogenicity islands (SPI) of the chromosome.

Among a small number of SPI described so far, SPI-1 and SPI-2 determine Type-III secretion systems (TTSS). This bacterium displaces bacterial effector proteins into the host cell cytosol upon infection via the SPI-1- encoded Type- III secretion system. Some of these proteins have the ability to bind to certain host enzymes like protein kinase. By this binding action, they can gain access to the epithelial cell cytosol, and, as an obvious phenomenon, they interfere with host cell signaling pathways while residing in the cell cytosol resulting in considerable changes in the host cell cytoskeleton, with consequent bacterial internalization and changes in host gene expression.

Studies have discovered that *Salmonella typhimurium* infection gives rise to cytoplasmic projections of the epithelial cells with characteristic disruption of the underlying cytoskeleton that facilitate the formation of ruffles mediating internalization of this pathogen into epithelial cells.

Salmonella infection also induces the production of inositol (isomeric alcohol) phospholipids in certain cell lines. Although it is unclear, these phospholipids may mediate calcium fluxes in the corresponding cells. In addition to these, this microorganism prompts the production of pro-inflammatory cytokines, principally IL-8, by inducing nuclear reactions eventually leading to cell disintegration. It has been reported that *Salmonella* can induce apoptotic macrophage death by the

activation of caspase-1 through binding of SPI-1 TTSS secreted protein SipB (sulphur-induced protein B) to it. Here, it will be worth focusing that, though apoptosis has always been demonstrated as a programmed cell death that does not elicit inflammation, introduction of caspase-1 in this mechanism, of course, induces the release of active pro-inflammatory cytokine, lL-1β.

Hence, the theory regarding apoptosis is deemed to be quite sophistic based on the observation of its diverse molecular mechanisms. Despite that research has revealed that *Salmonella* can affect abrupt dendritic cell death in a necrotic manner via SPI-1 TTSS pathway involving the so-called caspase-1 activation. For this reason, *Salmonella*-infected cell death in many cases can be possibly marked as pyroptosis for a clearer elucidation.

Yersinia enterocolitica

This gram-negative pathogen is fairly recognized as a causative agent of gastrointestinal ailment, septicemia and adenitis. The Type-III secretion system of *Yersinia* is determined on the virulence plasmids of this organism. The best studied secreted protein of this bacterium is the *Yersinia* protein tyrosine phosphatase (PTP) YopH (*Yersinia* outer protein H) (51-kDa), which is mandatory for *Yersinia* pathogenesis, and is translocated into eukaryotic cells by the Type-III pathway upon contact with the host cell. This protein has the capacity to activate antiphagocytosis or antagonize internalisation after the infection of epithelial cells or macrophages with *Yersinia*. Among other proteins characterized so far, YopE seems to be very significant in terms of antiphagocytic activity. It is largely accountable for cytotoxicity in host cells leading to drastic changes in cell morphology in collaboration with YopH. Investigations have reported that infection of epithelial cells or macrophages with *Yersinia* induces remarkable modifications in the microfilament structure of the host cell. For example, infection during progression reduces well-organized actin filaments to jumbled structures that become granular

in appearance. Manifestations related to cytotoxicity have been reported in host cells after infection with this pathogen. During this process, cells first undergo condensation, which is then followed by shrinkage in cellular structure.

YopH can also inhibit the oxidative burst in macrophages, which is adjudicated by ligation of Fc receptors, by its tyrosine phosphatase action. Another protein of this bacterium, YopP has been reported to be very effective being responsible for its contribution to apoptosis. lt blocks the activation of transcription factor NF-κB (Nuclear Factor κB) in macrophages by down-regulating the synthesis of antiapoptotic proteins, such as apoptosis inhibitors and Bcl-2 family members.

This phenomenon therefore, gives rise to the suppression of NF-κB -dependent antiapoptotic activities. Besides, YopP interferes with MAPK pathways by ligating and inhibiting MAPK kinase family members. However, the conception of upstream participation of FADD and caspase-8 in host cell apoptosis in response to *Yersinia* infection is still somewhat divisive. YopJ interferes with signaling pathways of host cells enhancing apoptosis and the pathogen kills macrophages without inducing inflammatory responses. YopK is another protein of *Yersinia* that helps the pathogen to evade pyroptosis.

Prospects and Constraints of a Cell Death

Death, as an ultimate recourse adopted by host cells responding to bacterial infection, is becoming a progressively interesting issue in the coverage of current medical studies. The reason why a cell undergoes termination via a set of molecular mechanisms is still debatable to a large extent. Undoubtedly, this natural scenario serves a broad range of subtle purposes of both the pathogen and host cells. It is now assimilated that apoptosis plays a vital role in T cell biology. Non-functional T lymphocytes, during development, as well as extravagant population of effector T cells, during immune responses, are deleted by apoptosis directly or indirectly that leads to the improvement of host cell immunity in terms of T lymphocyte

development. The dysregulation of the apoptotic process is believed to be the precursor of autoimmunity, tumorigenesis and immunodeficiency. It is also said that the two well known pathways of cell death, namely death receptor signaling and Bcl-2 coordination regulate T lymphocyte development and function.

Similarly, necrotic cell death helps with the eradication of disabled or unwanted cells, which could lead to physiological complications if not removed. Nevertheless, cell death may also be the reason for grave afflictions of the host. Massive amount of death will allow bacteria to further invade and gain access to target cells through tissues resulting in broad spectrum infections. For an instance, subversion of the autophagic pathway facilitates the pathogen with a suitable environment for huge reproduction inside the host cell tissue and supplies nutrients for its growth. Thus, bacteria largely benefit if host cells fail to survive due to invasion and subsequent induction of toxicity. Perhaps, infection is an essential task in the life cycle of all pathogenic bacteria. It may be assumed that they are committed to infecting the host to serve the purpose of better metabolism and effective cell division, which are much likely to be possible due to collapse of eukaryotic cells that are the natural reservoir of diverse growth factors for this bug. In contrast, it is actually challenging to understand the negative effects of cell death on this microorganism. Certain types of cellular demise are most likely to limit or down regulate bacterial population in the host body.

Autophagy or Type-II programmed cell death has been proven to be a specific mechanism in mammalian cells that is capable of degrading invasive bacteria. Likewise, apoptosis appears to be significant in circumscribing bacterial infection. For example, in Shigella-generated apoptosis, infection may subside due to the induction of a particular inflammatory response, which possibly plays a successful role in localizing the infection.

AEROBACTIN

Lecture nine

BY

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Iron acquisition

The indirect strategy for iron acquisition is based on a shuttle mechanism, which uses small-molecule compounds called siderophores as high-affinity ferric iron chelators, including the catecholates enterobactin, salmochelin, the hydroxamate aerobactin, and yersiniabactin. Salmochelin molecules were first discovered in *Salmonella enterica*. The *iroA* locus responsible for salmochelin production was also first identified in *Salmonella* spp. Salmochelins are C-glucosylated derivatives of enterobactin, encoded by the *iroBCDEN* gene cluster. Among *E. coli* isolates, *iro* sequences have been described in ExPEC strains isolated from patients with neonatal meningitis, UTIs, and prostatitis in humans, as well as from APEC isolates from poultry. Compared to enterobactins, salmochelins are superior siderophores in the presence of serum albumin, which may suggest that salmochelins are considerably more important in the pathogenesis of certain *E. coli* and *Salmonella* infections than enterobactins.

In ExPEC strains, the gene cluster responsible for salmochelin biosynthesis and transport is generally found on ColV or ColBM virulence plasmids, and has also been identified on chromosomal pathogenicity-associated islands (PAI) in some strains. The salmochelin gene cluster contains a gene encoding a cytoplasmic esterase, IroD. IroD can hydrolyze the ester bonds of both enterobactin and salmochelin molecules, which is required for subsequent iron release from salmochelin Aerobactin is a hydroxamate siderophore produced by most APEC strains and other pathogenic *E. coli*. It is synthesized by the *iucABCD*-encoded gene products and taken up by the *iutA*-encoded receptor protein. Despite the chemical differences among these distinct siderophores, each system is comprised of components mediating the specific steps required for ferric

iron uptake, including siderophore synthesis in the cytoplasm, secretion, reception of the ferri-siderophore at the outer membrane surface, internalization, and iron release in the cytoplasm.

Enterobacfin and Aerobactin

Enteric bacteria (Escherichia, Salmonella, Klebsiella and some species of Shigella) have been known since the early 1970s to produce a cathecol-type siderophore called enterobactin (also called enterochelin) under conditions of iron starvation in vitro. Enterobactin is also synthesized in vivo by virtually all clinical isolates of Escherichia coli and Salmonella, regardless of their origin and degree of pathogenicity. Enterobactin is therefore considered to be the 'native' or 'basal' siderophore of enterobacteria. In spite of its very high affinity for Fe(tlI), it is not clear whether enterobactin effects the virulence of strains which produce it. Failure to synthesize this siderophore impairs growth of Salmonella typhimurium (a typically invasive pathogen) in serum, but it is uncertain if intracellular virulence of this microorganism in mice is also altered. Involvement of enterobactin in both extra- and intra.cellular proliferation has not been definitively ascertained, but a number of considerations discussed below argue against it.

Distribution of Aerobactin Production among Enterobacteria The ability to produce aerobactin is widely distributed among the Enterobacteriaceae. At present, no evidence of aerobactin synthesis by a family other than enterobacteria has been published. Regardless of the origin of the isolates, the genera Escherichia, Shigella andEnterobacter demonstrate ahigh incidence of aerobactin-positive strains, typically more than 40%. However, in isolates of other enterobacterial genera, i.e. Klebsiella, Citrobacter, Proteus, Morganella, Yersinia, Serratia and

Salmonella, aerobactin production is less common (< 20%). Shigella, which usually synthesize only aerobactin, and Enterobacter (66) are the genera in which this siderophore is present with the highest incidence. Although aerobactin genes were first found contained on pColV plasmids, they have also frequently been detected in conjugative resistance plasmids and/ or plasmids, often belonging to the FI incompatibility group, carrying other virulence determinants.

In addition to plasmids, aerobactin has also been found in many instances to be chromosomally encoded. At present the significance, if any, of either plasmid or chromosomal localization for virulence is unclear. Regardless of the origin and location of aerobactin genes, the same organization of the operon has been detected by DNA hybridization in virtually all aerobactin-producing strains. The mechanism by which aerobactin genes have been distributed among enterobacterial genera and their replicons (chromosome or plasmid) is uncertain, although it is possible that the whole system behaves as a transposon or transposon-like unit.

Aerobactin as a Virulence Factor

Two approaches have been used to assess the contributory role of aerobactin to bacterial virulence, namely experimental infection systems and statistical studies on the epidemiology of aerobaetin production. Obviously, the first type of analysis sheds more light on the mechanism by which aerobactin contributes to the infective process. However, the epidemiological reports largely outnumber the studies in well-defined model systems.

Experimental Infections

A limited number of studies have been published in which the contribution of aerobactin to a particular type of infection has been determined by exposing animals or cell cultures to isogenic enteric strains which differ only in their ability (Iuc^+ phenotype) or inability (Iuc^-) to produce aerobactin. The pioneering investigations which identified the pColV-encoded iron transport system as a virulence factor used this approach. However, only in the cases of invasive strains of E. coli and K. pneumoniae, both extracellular pathogens, has the contribution of aerobactin to virulence been unequivocally proven. In a study involving Klebsiella pneumoniae K1 and K2, the most common serotypes of virulent Klebsiella, it was demonstrated that an increase in the virulence of these strains after they had acquired a plasmid encoding aerobactin production by measuring the intraperitoneally-administered 50% lethal dose in mice. Indeed, a precise correlation was observed between the degree of Klebsiella pneumoniae-induced pathogenicity and synthesis of the siderophore.

Epidemiological-Studies

The analysis of the frequency of aerobactin production among isolates from different types of infections has been the most common method to determine whether the siderophore plays a role in bacterial virulence. This type of data (with respect to the conclusions drawn) should be cautiously evaluated. Although the literature often shows contradictory conclusions, there are some definite trends. Most epidemiological studies have been carried out with Escherichia coli strains derived from several sources, namely blood, faeces and urine. In all cases, the incidence of aerobactin production is very high, but the actual figures of the frequencies of

occurrence demonstrate substantial differences depending on the year and the geographic location of the study in question A surprisingly high percentage of aerobactin-producing E. cell strains are found in the urine of patients with pyelonephritis and cystitis. Aerobactin has often been associated with other virulence determinants in strains causing these diseases. An important finding in isolates from patients with urinary tract infections is that aerobactin production is correlated with certain O:K:H serotypes, and production of the siderophore in isolates of the same serotype is independent of the clinical origin. The association of aerobactin production with a particular serotype has also been described for clonal groups of human invasive strains E. coil K1. in the case of pyelonephritis, the O:K:H serotype is associated not only with aerobactin, but also with adherence factors, resistance to serum and hemolysin production.

LIPOOLIGOSACCHARIDES

Lecture ten

BY

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Meningococcal LOS interacts with human cells, resulting in the production of proinflammatory cytokines and chemokines, including interleukin 1 (IL-1), IL-6, and tumor necrosis factor (TNF), that are important in the pathogenesis of meningococcal disease. While pili and Opa and Opc outer membrane proteins are also critical, LOS is one of the structures important in mediating meningococcal attachment to and invasion into epithelial cells. The role of LOS in these events is further substantiated by the findings that LOS-deficient meningococcal mutants show impaired adherence and reduced induction of serum cytokines compared to the wild-type strain.

LOS molecules consist of three oligosaccharide chains attached to a lipid A core . The oligosaccharide chains of LOS are similar in sequence and linkage with the oligosaccharides expressed on the surface of human cells 26. The synthesis of these compounds requires a series of glycosyl transferases.

Besides their highly conserved core structures, the terminal oligosaccharides of LOS molecules undergo rapid phase variation . LOS variation is mediated by a change in the number of guanines in the middle of the coding sequences of several key enzymes, which results in alterations in the expression of these glycosyl transferases and the surface expression of various LOS isoforms. At any given time, several LOS structures with varying terminal oligosaccharides may be expressed on the outer membrane of *N*. *gonorrhoeae* .

Because LOS structures can vary in strains, it has been difficult to study the structure and function of LOS. Early studies that used the male challenge model indicated that LOS was involved in host cell invasion. It was shown that the lacto-*N*-neotetraose LOS structure can inhibit the invasion of HEC-1-B cells by gonococci. Using cultured hepatoma cells as

a model system, it was identified the asialoglycoprotein receptor as the binding site of LOS-containing lacto-*N*-neotetraose, and showed that gonococci increased the expression level of the asialoglycoprotein receptor in the hepatoma cells. The asialoglycoprotein receptor is also expressed in human urethral epithelial cells.

Furthermore, terminal galactose residues on LOS and elsewhere are decorated with sialic acid, which blocks antibody binding, activation of complement, phagocytosis, and intracellular killing. Therefore, antigenic mimicry of host antigens is an important defense mechanism provided by the oligosaccharide component of the LOS to avoid innate and adaptive host defense mechanisms.

However, some strains of *H. somni* isolated from the bovine genital tract, particularly the normal bovine prepuce, are incapable of LOS phase variation, sialylation of the LOS, and expression of ChoP. At least 1 such strain has been shown to be avirulent, underscoring the importance of the LOS as a virulence factor, although this strain is deficient in other factors as well.

The structure and arrangement of the inner core glycoses (heptose and 3-deoxy-D-*manno*-2-octulosnic acid) is remarkably similar to the inner core oligosaccharide on some strains of *Neisseria* spp., and mutants that contain a truncated LOS oligosaccharide are considerably more serum-sensitive than the parent strain. Therefore, the LOS is a critical component that enables *H. somni* to resist host defenses and cause disease.

Lipooligosaccharide (LOS), the lipid A-containing structure in the outer membranes of Neisseriae (including the gonococcus), has a

relative paucity of glycosylation when compared to lipopolysaccharide of enteric Gram-negative species.

LOS variation has been implicated in antigenic variation and escape from immunologic surveillance, resistance to complement and other host antimicrobial factors, and bacterial adherence to host substrate. Variations in gonococcal LOS are largely due to phase-variable expression of genes involved in LOS biosynthesis, those in the *lgtABCDE* operon and *lgtG*, which determine core oligosaccharide structure and *lst*, which determines LOS sialylation.

Sialylation of LOS facilitates binding of complement regulatory molecule, factor H, inducing high levels of complement resistance. Gonococcal LOS sialylation also helps it resist killing by polymorphonuclear leukocytes. Gonococci lacking the *lst* gene have reduced virulence in murine models of gonorrhea, and LOS from gonococci isolates from human urethral exudate is highly sialylated, suggesting that LOS sialylation is important in pathogenesis. Phosphoethanolamine decoration of the oligosaccharide and lipid A moiety is an additional structural modification of gonococcal LOS that is mediated by the *lpt3*, *lpt6*, and *lptA* genes, respectively.

Structural changes in LOS are mediated by phase-variable expression of many of these biosynthetic genes. The interaction of gonococcal LOS with the human asialoglycoprotein receptor (ASGP-R) allows entry into primary human urethral epithelial cells. Gonococcal LOS is also known to interact with Toll-like receptor-4, dendritic cell-SIGN (CD209), and the inflammasome to activate inflammatory signaling in host immune cells. The potency of immune signaling activation has been correlated in some cases to variations in LOS structure. The role of variability in LOS-induced inflammatory signaling remains to be determined.

N. gonorrhoeae possesses a lipooligosaccharide (LOS) anchored in the outer membrane through lipid A, with short, triple-branched, and variable glycan chains. These depend on glycosyltransferases some of which are subject to off–on expression through phase variation determined by a slip–strand mispairing mechanism resulting from poly-G tracts in the coding sequences of the genes .

However, some of the oligosaccharides mimic host glycolipid epitopes. In addition, LOS can be sialylated by means of a gonococcal sialyltransferase for which the host supplies the substrate, cytidine monophosphate-*N*-acetylneuraminic acid. Some, though not all, of the glycan subunits are immunogenic, and antibodies to them can induce complement-dependent bacteriolysis. However, sialic acid residues on LOS interfere with this bactericidal activity by facilitating the binding of the complement regulatory factor H . Other LOS glycan structures enhance resistance to complement by binding the regulatory protein, C4b-binding protein .

Gonococcal LOS is highly endotoxic, probably through interaction with Toll-like receptor (TLR) 4 and CD14, and contributes significantly to the inflammatory response induced by gonococcal infection and the resulting damage to tissues such as the fallopian tube . However, LOS is not considered a prime candidate for a vaccine antigen unless its toxicity can be reduced or eliminated. In addition, carbohydrate antigens are usually T-independent unless coupled to proteins. Moreover, sialylation of LOS inhibits the bactericidal activity of complement-fixing antibodies against it.

Because LPS is essential for the viability of most Gram-negative bacteria, components of the lipid A biosynthetic pathway are emerging targets for the development of new broad-spectrum antibiotics. One such

enzyme is LpxC, a zinc-dependent cytoplasmic deacetylase that catalyzes the first committed step in lipid A biosynthesis.

Gene disruption experiments revealed that this enzyme is essential in *Escherichia coli*, and the first reported LpxC inhibitors displayed promising antimicrobial activities against *E. coli* . CHIR-090, a newer small-molecule inhibitor of LpxC with low nanomolar affinity, is as effective against Gram-negative pathogens as the DNA gyrase inhibitor ciprofloxacin.

Structural and biochemical analysis have further revealed that the amino acid side chains in LpxC that are critical for substrate binding and catalysis are involved in the binding of CHIR-090. These studies provided a template for the development of more potent LpxC inhibitors with a wider spectrum of antimicrobial activity. Based on CHIR-090 interactions with hydrophobic substrate-binding passage in *Aquifex aeolicus* LpxC, and on the molecular analysis of CHIR-090 resistance of the *Rhizobium leguminosarum* LpxC, two biphenyl diacetylene-based compounds (LPC-009 and LPC-011) with enhanced activity against LpxC were generated.