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Porins in the Inflammatory and Immunological Response Following *Salmonella* Infections

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1. Introduction

Today numerous information are available on the molecular mechanisms activated by *Salmonella* and its components during the interaction with host cells and in determining the disease state. The molecular mechanisms of how *Salmonella* enter host cells and function as an intracellular pathogen are under intense investigation. Much progress has been made in identifying the bacterial factors that mediate invasion. Once *Salmonella* enters a cell, it remains within a membrane-bound vacuole and does not appear to fuse with lysosomes, the outcome of infection is determined both by bacterial and host factors, including the virulence of *Salmonella* strain, and the ability of the host to respond with an inflammatory and immunological reaction. The host response involves multiple cells that are resident at the site of infection or infiltrate from the circulation. Induction of an array of cytokines occurs in response to infection of macrophages with live *Salmonella* and after exposure to various *Salmonella* components including lipopolysaccharide (LPS), and porins. Of the different biologically active components present in Salmonella, LPS and porins are the most potent inducer of host response. LPS and porins present an intrinsic biological activity on cell involved in the inflammatory response and also on other types of cells; moreover they are immunogenic molecules against which the organism raises the humoral and cellular response. The LPS molecule is the most studied. Techniques previously used in the extraction of LPS from the endotoxin had greatly favoured the study of this portion of the macro-complex ignoring the protein fraction, allowing the identification of most of the effects of endotoxin with those of LPS. Subsequent extraction techniques for membrane proteins then allowed the study of the protein fraction, which was extracted globally in the endotoxin. Later experiments suggested that a chemical subunits of LPS, lipid A, was the actually toxic moiety and that the O-specific chain found on LPS was not involved in the toxic effect. The endotoxin-associated protein (EP) consists of a complex of 4-5 major proteins that range in size from 10 to 35 KDa. Originally considered to be a superfluous carrier of LPS, EP is now recognized to have potent biological activities, some of which are unique (Mangan et al., 1992). For example, EP is a

powerful mitogen for C3H/HeJ mouse and human lymphocytes which are hyporesponsive to LPS. Among the proteins associated with LPS, the techniques actually used for the extraction of native proteins from the cellular membranes allowed the isolation of outer-membrane proteins (OMPs). LPS and OMPs are released by different bacteria during both in vitro and in vivo growth and this release is significantly enhanced when the bacteria are lysed following exposure to antibiotics or human serum. Molecular complexes of LPS and OMPs, together with the other molecules which constitute the external surface of Gram-negative bacteria, are released as outer membrane vesicles (OMVs). OMVs are formed by blebbing and pinching of segments of the bacterial outer membrane.

2. Outer membrane and porins

The cell envelope of Gram-negative bacteria (Figure 1) is composed of two distinct membranes, the inner plasma membrane and the outer membrane. The peptidoglican layer is located between the two membranes and this area between the plasma membrane and the outer membranes is referred to as the periplasmic region. The outer membrane is composed of phospholipid and protein as is the cytoplasmatic membrane. The outer leaflet is occupied by about 45% lipopolysaccharide (LPS). The phospholipid is localized almost exclusively in the inner layer of the outer membrane bilayer. In *Salmonella enteric serovar Typhimurium,* the phospholipid composition of the outer membrane consists predominantly of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin. LPS consists of a hydrophobic membrane anchor, lipid A, a short core oligosaccharide, and an O antigen that may be a long polysaccharide. The lipid A is rather well conserved among Gram-negative bacteria. The core oligosaccharide and O-antigen, if present, is the most variable part of LPS and shows even a high degree of variability between different strains of the same species. In bacteria the number of porin copies was determined to be up to 100.000 for cell. Porins form β-barrels with 14, 16 or 18 strands, all of which are connected by extraplasmic loops and periplasmic turns with the particularly long loop L3 folded inside the barrel. Porins of 16 strands are called general or non-specific porins and form pores allowing the diffusion of hydrophilic molecules, showing no particular substrate specificity; while 18-strands porins are substrate specific porins. The best-studied examples is the sucrose-specific porin ScrY from *S. typhimurium*. The three-dimensional structures of this specific porin has been elucidated, (Forst et al., 1998). ScrY forms homotrimers whose monomers consist of 18-stranded antiparallel β-barrels. As was found with the general porins, the third loop, L3, folds back inside the β-barrel. A peculiar feature of ScrY is the presence of a 70-residue-long N-terminal extension, which hangs out into the periplasm in the form of a parallel triple-stranded coiled-coil.

An homology based 3D structural model for the porin OmpC from *S. typhimurium* was built to understand the possible unique conformational features of its antigenic loops with respect to other immunologically cross reacting porins. The homology model was built based on the known crystal structures of the *E. coli* porins OmpF and PhoE. The resulting model was compared with other porin structures, having β-barrel fold with 16 transmembrane β-strands, and found that the variable regions are unique in terms of sequence and structure (Arochiasamy et al., 2000).

Recently, a structural model for a 50kDa antigen protein of *S. enterica serovar Typhimurium* was also built by Siew-Choong et al. (Yee et al., 2011). The characteristic of the built model also resembles the structure of known transmembrane proteins in other Gram-negative bacteria (S.

Galdiero et al., 2003). It shows a similar structure as the TolC transmembrane channel protein with the combination of β-barrel domain projecting from the membrane, across the periplasmic space with α-helical domain and the equatorial domain (mixed of β-sheets/α-helices). The upper part of the structure is open and could provide solvent access, while the lower part is narrowed. The structure shows that it may be an ion channel whose conductance depends on the open or close conformation at the lower end, which is similar with the characteristic of TolC and its analogues. The surface exposed loops might act as a "lid" to access into the top end of the β-sheets domain. The β-barrel domain consists of 16 strands, which is within the number of strands that has been characterized for other bacterial outer membrane proteins (S. Galdiero et al., 2007). The 40 Å long axis of the β-barrel domain fits into a lipid bilayer membrane which is typically 30 Å. As for other porins the base of the β-barrel is mainly composed of aromatic residues, specifically phenylalanine and tyrosine. These residues are usually found in a typical β-barrel membrane protein to define the inner edge of a lipid bilayer of membrane. The lower part of the built structure is the left twisted antiparallel-helices barrel, which is involved in the control of the opening or closing of the access.

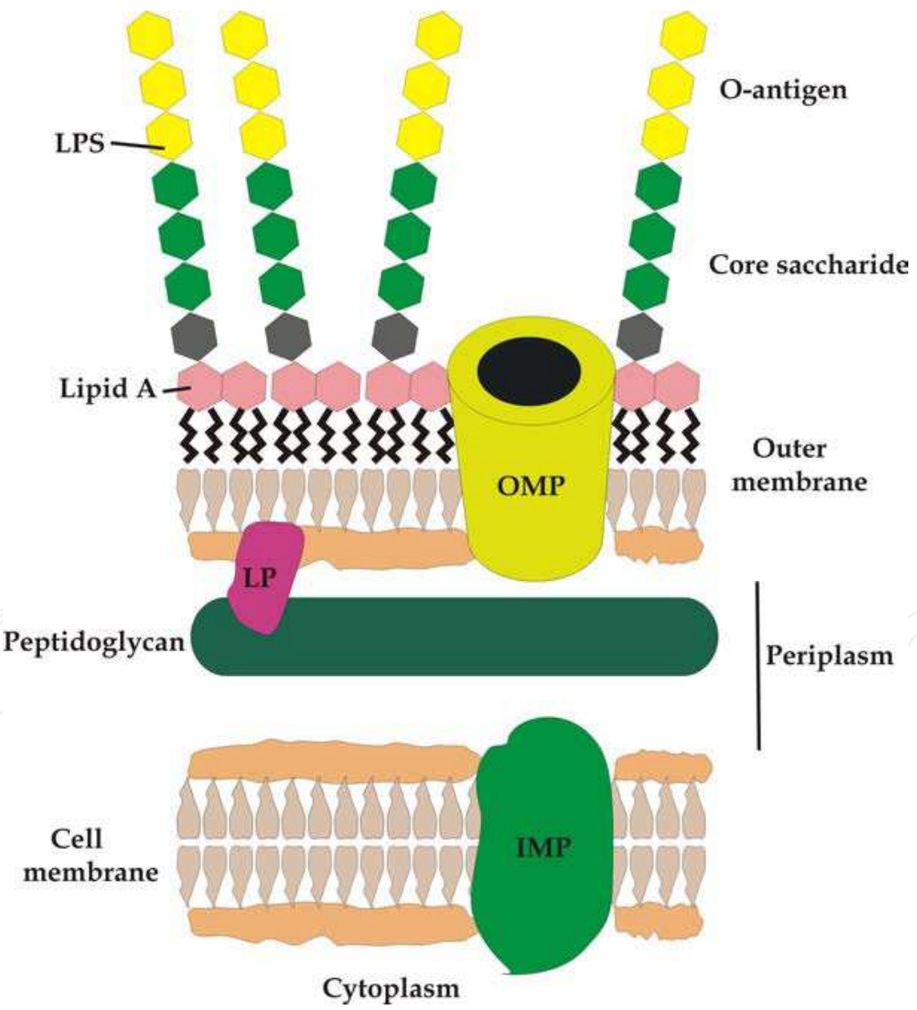

Fig. 1. Depiction of Gram-negative cell envelope. IMP, integral membrane protein; LP, lipoprotein; LPS, lipopolysaccharide; OMP, outer membrane protein.

3. Biological activity of porins

The release of porins at the infection site, whether secreted during growth or derived from the lysis of the bacterial cells, involve the host defence and influence the course of enterobacterial diseases.

Porins of *S. enterica serovar Typhimurium* added to macrophage cultures in vitro are able to modify several macrophage functions. The superficial hydrophobicity of macrophages adhering to the slide, as measured by the average contact angle monolayers, shows an increase of about 15-20%. The phagocitic index of macrophage treated with porins was found to be significantly lower. The same reduction was observed in the intracellular killing of macrophages treated with porins (Tufano et al., 1984). Porins inhibit phagocytosis by activating the adenylate cyclase system (Di Donato et al., 1986). The effect of *Salmonella* typhimurium porins was also studied on human polymorphonuclear leukocytes (PMNs). Labelled porins were shown to bind to the PMNs, and could be completely displaced by unlabeled porins. The binding caused modifications of membrane integrity and of the physico-chemical characteristics of the PMN surface, e.g. decreased oxidative burst, decreased hydrophobicity and altered cell morphology. The porins acted as both chemotaxins and chemotaxinogens. When PMNs were preincubated with porins their migration in the presence of commonly used chemoattractions (serum activated by zymosan or N-formyl-L-methionyl-L-leucyl-L-phenylalanine) was inhibited (Tufano et al., 1989).

S. enterica serovar Typhimurium porins injected into the paws of rats, induced a dose dependent edema which was maximal at 2 to 3h and still present at 5h. Edema was unaffected in animals which had their complement levels depleted, demonstrating that inflammation was not associated with complement activation; however it could be decreased by indomethacin and by dexamethasone. Rat peritoneal cell incubated with porins released histamine but little prostacyclin, suggesting that porins have little ability to induce the prostenoid producing enzyme cicloxygenase II (F. Galdiero et al., 1990).

Porins were also shown to kill D-glucosamine-sensitized LPS-responsive and LPS-unresponsive mice. A 100 μg amount of porins was sufficient to kill 80-90% of animals. But lethal effect of the porin preparation could be completely blocked by pre-administration of a neutralizing antiserum to TNF-α but was not abolished by polimixin B indicating that LPS did not contribute to the biological responses. Porins were also pyrogenic in rabbits and elicited a localized Swartzman reaction when used as the sensitizing and eliciting agent (F. Galdiero et al., 1994).

4. Porins interaction with host cell membranes and signal transduction

The cell ability to sense external stimuli and to react by initiating a program of expression often involves propagation of a cell surface-initiated signal along a specific pathway of protein kinases whose ultimate targets are nuclear transcription factors (Figure 2). These pathways consist of a cascade of biochemical events that include phosphorylation of a variety of kinases, that in turn modulate other factors that control gene expression. Porins purified from *S. enterica serovar Typhimurium* induce tyrosine-phosphorylation in THP-1 cells and in C3H/HeJ macrophages. After porin stimulation a pattern of tyrosine-phosphorilated proteins appeared in the soluble cytoplasmic fraction, the membrane fraction and in the

insoluble protein fraction. The events of tyrosine protein phosphorylation were present in macrophage from LPS-hyporesponsive C3H/HeJ mice stimulated with porins, while they were markedly reduced where the macrophage where stimulated with LPS-(R). Among the most prominent tyrosine phosphorylated bands in porin-stimulated cells there is a number of proteins with a molecular mass that is similar to that of the family of tyrosine/serine/threonine protein kinases. Mitogen-activated protein kinase (MAPK) cascade are among the best known signal transduction systems and play a key role in the regulation of gene expression as well as cytoplasmic activities.

MAPKs have been shown to be involved in the regulation of cytokine responses. MAPKs are activated upon phosphorylation of both tyrosine and threonine residues by MAPK kinase (MEK). These enzymes participate in cell signalling pathways leading to AP-1 and NF-κb activation following porin stimulation of cells. Raf-1 was also phosphorylated in response to the treatment of U-937 cells with porins; the porin-mediated increase in Raf-1 phosphorylation is accompanied by the phosphorilation of MAPK kinase 1/2 (MEK 1/2), p38, ERK 1/2 and C-Jun N-terminal kinase. P38 signalling pathway mainly regulates AP-1 and NF-κb activation in cells treated with *S. enterica serovar Typhimurium* porins. The transcriptional factor AP-1 is composed of multiple protein complexes formed between the protein products of proto-oncogenes C-fos and C-jun and their related gene family members. The fos family consists of C-fos, the gene for Fos-related antigen-1 (Fra-1), Fra-2 and Fas-B and its naturally truncated form Fas B2; the Jun family consists of C- jun, Jun B and Jun-D. The AP-1 family of transcription factors consists of homodimers and heterodimers of these subunities; the different complexes regulate their abilities to transactivate or repress transcription. AP-1 composition may change in the cell as a function of time and stimules; the binding affinity for a given target DNA sequence is determined by the different AP-1 dimer combinations and the context of the surrounding sequences. In U937 cells treated with porins from *Salmonella*, different complexes including C-Jun and Fra-2 subunits appeared. While in cells treated with LPS, the stimulus leads to AP-1 complexes containing Jun D, C-Fos and C-Jun, stimulation by porins induces AP-1 complexes containing Fra-2 in addiction to the other subunits (M. Galdiero et al., 2002). The formation of different complex represent a further difference between stimulation with LPS and stimulation with porins to be added to other observations: that cytokine release after stimulation with porins begins after 120 min and continues for 5 to 6 hours, whereas cytokine release following LPS stimulation begins after 30 min. and decrease at 120 min (M. Galdiero et al., 1995).

Porins trigger multiple synergistic signal transduction pathways, including protein kinase A (PKA), proteine kinase C (PKC), NT proteine tyrosine kinase (NT-PTKs). The role of PKC in signal transduction in mouse macrophages stimulated by *S. enterica serovar Typhimurium* porins is reported by Gupta S et al., (Gupta et al., 1999). Their experiments showed that porin activation of macrophages results in the increased inositol-triphosphate and intracellular Ca2+ mobilization: there is a translocation of PKC to the membrane which is accompanied by nitric oxide release.

Several polypeptide ligands use the JAK-STAT molecules in signal transduction (Darnell et al., 1994). The family of transcription factors called STAT (signal transducers and activators of transcription) have been found to be activated by the Janus Kinases (JAKs) that are associated with the cytokine receptor components (Kisseleva et al., 2002) . In resting cells the

STATS, when phoshorylated by the JAKs, dimerize via their SH2 domains and traslocate in the nucleus, where they interact with specific DNA sequences and transactivate the associated genes. The Jak/Stat signalling pathway plays a fundamental role in response to infection and in sepsis (Scott et al., 2002) In vitro experiments on U937 cells demonstrate a complex indirect mechanism of STAT-1 and STAT-3 activation after stimulation of cells with porins. The treatment with porins did not results in increase of JAK phosphorylation although STAT-1 and STAT-3 activation was observed. The activation of STAT-1/STAT-3 by porins can occur through the activation of MAPK and possibly other PTKs but not through JAK activation (M. Galdiero et al., 2006).

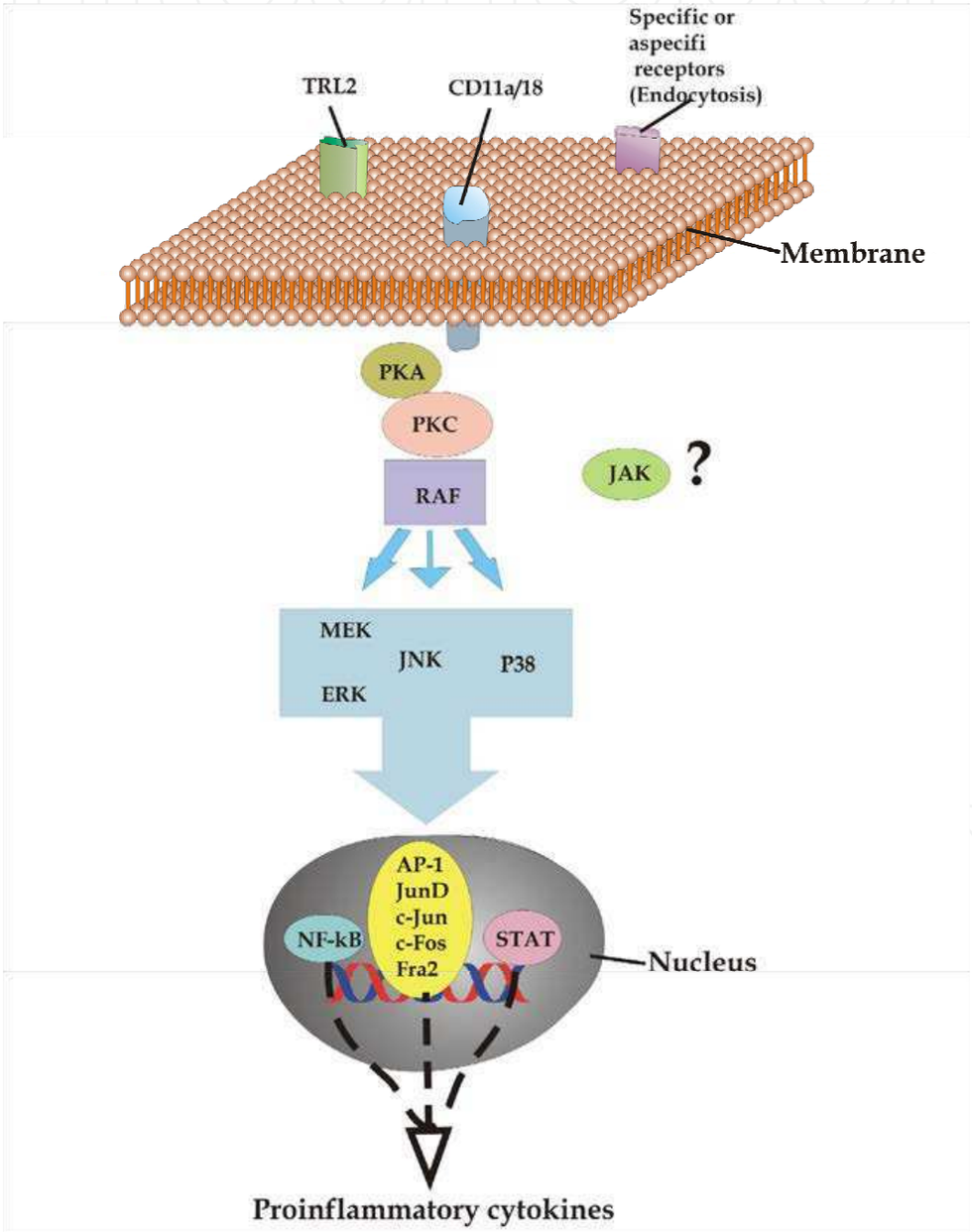

Fig. 2. Porin signal transduction pathways

5. Cytokines release by porins

Many of the pathophysiologic mechanisms of Gram-negative bacterial infections are due to bacterial surface components acting on cells directly or via mediators such as cytokines. Cytokines are polypeptides that exert a wide spectrum of biological effects, including haematopoietic, metabolic, inflammatory and immunologic homeostasis. Among the large family of cytokines, porins by *S. enterica serovar Typhimurium* induce the release of TNF-α, IL-1, IL-6, and TGF by macrophages and IL-4 and IFN-γ by lymphocytes. The role played by porins in the production of cytokines derives from the comparison with LPS-S e LPS-R extracted by the same strain of *S. typhimurium*. Porins at 1 μg/ml induce the greatest release of TNF-α, IL-1α and IL-6 by monocytes and IL-4 by lymphocytes, while porins at 5μg/ml induce the greatest release of IFN-γ by lymphocytes. The R-form of LPS (LPS-R) induces the greatest release of TNF-α and IL-1α by monocytes when used at 1 μg/ml concentration. At concentration of 5 and 10 μg/ml, respectively, LPS-R induce the maximal release of IL-6 from monocytes and the maximal release of IL-4 from lymphocytes. The S-form of LPS (LPS-S) induces the greatest release of TNF-α, IL-1α and IL-6 by monocytes and that of IL-4 by lymphocytes when used at a concentration of 1 μg/ml. Porins (5 μg/ml) induce the release of IL-8 by THP-1 cells after 24h of stimulation (Vitiello et al., 2004). The level of IL-8 in THP-1 cells stimulated with porins was comparable to that induced in response to 1 μg/ml of LPS-R.

While CD-14, CD-11/18 and Toll receptors 2 and 4 appears to be very important LPS signal transducer, porin-specific receptors are still unknown. Therefore, it is possible that porin stimulation is not due to binding to specific receptors, but the consequence of the perturbation of the cell membrane lipoproteic phase, induced during adsorption or porin penetration. CD-14 is a glycosyl-phosphatidyl inositol linked 55 kDa protein present on the surface of monocytes and polymorphonuclear leucocytes, and it function as the cell surface receptor for LPS and several surface components of Gram-positive bacteria. CD14 is also found as a soluble protein (sCD14) in human serum. CD14 lacks transmembrane and cytokine-binding domains and is not believed to have intrinsic signalling capabilities. Toll-like receptors 4 (TLR4) appears to be very important LPS signal transducer. It's thought that, also, Toll-like receptor 2 (TLR2) functions as a signal transducer upon LPS binding by CD14. TLRs make up a family of evolutionary conserved pattern recognition molecules that are important signal transducers for the induction of mammalian innate immunity responses, including cytokine responses. The best characterized TLRs to date are TLR2 and TLR4. TLR2 is involved in the recognition of a wide assay of bacterial products, including peptidoglycan, lipopeptides, zymosan and bacterial lipoproteins, whereas TLR4 is activated by LPS. CD 14 acts as a abroad specificity coreceptor that can enhance cell activation induced by TLR4 or TLR2 agonists. Data from *Haemophilus influenzae* (Hib) porin and from neisserial porin P or B indicate that porins from different bacteria may be recognized by TLR-2 (M. Galdiero et al., 2004). The Hib porin-induced TNF-α and IL-6 production was eliminated in macrophages from TLR2 or MyD88 deficient mice. In contrast, macrophages from LPS hyporesponsive C3H/HeJ mice which are defective in TLR4 function, responded normally to Hib porin. Neisserial porin adjuvant activity was mediated by surface expression of B7-2 and class 2 major histocompatibility complex on B cells by TLR-2-dependent mechanisms; the presence of the adaptor molecule MyD88 was also required.

CD11/18 (M. Galdiero et al., 2004) integrin may also participate in LPS signalling. This family of receptors are heterodimeric cell surface glycoproteins composed of a CD11 and a

CD18 subunit. The release of TNF-α, IL-6 and IL-8 by THP-1 cells stimulated by porins is independent of CD14, but is partially dependent on CD11/18 integrins. *S. enterica serovar Typhimurium* porins enhance the synthesis and release of IL-6 in U937 cells regulating the transcriptional activity of IL-6 gene by nuclear transduction of NF-κB. The characterization of the human IL-6 promoter revealed a highly conserved control region of 300bp upstream of the transcriptional initiation site that contains the elements necessary for its induction by a variety of stimuli commonly associated with acute inflammatory or proliferative states. In particular, electrophoresis mobility shift assay, as well as promoter deletion and point mutation analysis, revealed the presence of an NF-κB binding element. In U937 cells stimulated by *Salmonella* porins, NF-κB is able to enhance IL-6 gene promoter activity. Activation of this nuclear factor may be responsible for porin induced expression and release of IL-6 (Finamore et al., 2009).

These observations allow to outline a specific ability of porins that is expressed by stimulation of the cell surface, signal transmission, activation of nuclear factors, activation of gene promoters and finally release of cytokines.

6. Transmigration of leukocytes following porin activation

It has long been recognized that *Salmonella* provokes un intense intestinal inflammatory response, consisting largely of neutrophil migration across the epithelial lining of the intestine (M. Galdiero et al., 1999); this inflammatory event manifests as an epithelial dysfunction, namely diarrhea. In an in vitro model, Mc Cornick et al (McCormick et al., 1995) showed that *S. typhimurium* tran-epithelial signaling to polymorphonuclear neutrophils (PNM) plays a direct and substantial role in stimulating enteritis in humans. Leukocyte-endothelial cell interaction both in vivo and in vitro are active multistep processes. The initial adhesion of circulating leukocytes to vascular endothelium is induced by interaction of constitutively functional leukocyte homing receptors with regulated endothelial cell ligands. During inflammation a dramatic increase of endothelial cell surface molecule expression occurs that support the adhesion of circulating leucocytes. Bacteria or bacterial products may constitute important inducers of surface molecule expression on endothelial cells. LPS-S induce adhesion of leucocytes to endothelial cells as potent as that induced by IL-1β (Takeuchi et al., 1967) *S. typhimurium* porins and LPS-R are able to induce the release of s-E-selectin and sICAM-1 from human umbilical vein endothelial cells (HUVEC) and also were to up-regulate the surface expression of E-selectin and ICAM-1 on endothelial cells (Donnarumma et al., 1996).

Treatment of the HUVEC with either porins or LPS in the form S or R increased the transmigration of different leukocyte populations, in particular that of neutrophils; transmigration increased remarkably during the simultaneous stimulation of endothelial cells by IL-1β together with either porins or LPS (M. Galdiero et al., 1999). Porin treatment caused transmigration that lasted several hours longer than that caused by LPS and further increase the activation of those cells already activated by IL-1β. Consequently, the in vivo activity of the two molecules shows an effect prolonged in time. In vitro, the simultaneous stimulation of endothelial cells with IL-1β and either porins or LPS causes overlapping effect leading to a very high migration index. Neutrophil transmigration was partially inhibited by monoclonal antibodies (MoAb) binding to E-selectine; the transmigration of lymphocites and monocytes was partially inhibited by MoAbs anti-VCAM-1; the transmigration of

neutrophils, lymphocytes and monocytes was partially inhibited by MoAb anti-ICAM1. Monocyte and granulocyte transmigration was, also, inhibited by MoAbs binding to CD11a/CD18 and CD11b/CD18. Lymphocyte transmigration was inhibited by MoAbs CD11a/CD18 and not by CD11b/CD18. Therefore, porins may constitute important inducers of surface molecule expression on endothelia cells. This ability makes these molecules particularly important in the inflammatory process during *Salmonella* infections.

7. Porin immunogenicity

Porins demonstrate immunogenic and adjuvant properties. Heat denaturable surface components play a role in inducing protection to *S.enterica serovar Typhimurium* infection in mice (Isibasi et al., 1994). The protective role of the outer membrane proteins or of porins from Neisseria (Melancon et al., 1983), *Salmonella* (Muthukkmar et al., 1993), Haemophilus and Vibrio genus has been shown. Antiporin antibodies have been demonstrated to be bactericidal and opsonic (Isibasi et al., 1994); patients with pelvic inflammatory disease (PID), who recover spontaneously have high levels of antiporins antibodies showing that the presence of serum antibodies to neisserial porins may correlate with protection against PID. Several studies have been recently performed with porins extracted from *N. meningitidis, N. gonorrhoeae* and *H. influenza* (Massari et al., 2003; Song et al., 1998; Wetzler et al., 1996). Porin vaccines have also been developed to protect against *S. typhimurium* infection. Porins are excellent antigens, efficiently stimulating humoral and cell-mediated immune response of the host immune systems which could play a role in the protection against the disease. *S. enterica serovar Typhimurium* porins are also able to induce expression of CD86 on antigen–presenting cells (Massari et al., 2002). Macrophages from mice immunized with porins and infected later with Salmonella, express 53% more B7 versus control macrophages and can therefore support a host–protective immune response.

Complement is an important arm of innate immune defenses against invading pathogens. Complement activation leads to the deposition of C3 fragments, which can enhance opsonophagocytosis of microbes. Porin contribute to complement activation mainly through the classical pathways in an antibody-indipendent manner. All of the porins tested to date have been shown to bind fragment C1q, the first component of the classical pathway of the complement system. The effect of porins purified from *S. enteric serovar Tiphimurium* on the complement system was investigated both in vitro and in vivo. Incubation of porins with either human or guinea pig serum resulted in the consumption of the total complement activity when an amount of porins ranging from 8 to 10 μg per 100 ml of serum was used. The activation of complement system was temperature dependent, suggesting an active process rather than passive adsorption of the complement components by porins. In addition, the activation had a fast kinetic and proceeded mainly through the classical pathway. This conclusion is supported by the consumption of C1s and C4 in normal human serum treated with porins and also by the depletion of C3 activity in the C1s-deficient serum which was marked only when purified C1s was added to the serum before incubation with porins. Injection of 100 μg of porins into guinea pigs induced profound complement consumption at 6 h post-injection that persisted up to 12 h (F. Galdiero et al., 1984). Also porin Omp K 36 from *Klebsiella pneumonia* interact with C1q.

The cloning of the genes encoding the outer membrane proteins has facilitated the production of pure porins free from other bacterial antigens for investigation as potential

products for protective responses. The protection against bacterial infection is correlated to the presence of the antibodies with the ability to activate complement mediated killing of bacterial cells. The amminoacid sequences of the superficial loops of porin are highly variable in different strains and are the regions actually responsible for stimulation the production of bactericidal antibodies (Snapper et al., 1997). The role of surface loops of porin in immunological responses has been also studied in Haemophilus. Protein P2 of H. influenzae is a homotrimeric porin, which constitutes approximately one-half of the total outer membrane protein and it is an important target of the immune response to Haemophilus. P2 contains 16 transmembrane regions with β-sheet conformation and 8 suface-exposed loops. Analysis of sequences of P2 genes indicates that the transmembrane regions are relatively conserved among strains while considerable heterogenicity exists in surface-exposed loops (Qi et al., 1994). Challenging with whole bacterial cells resulted in a prominent antibody response directed at the P2 molecule. Analysis of the antibodies to whole organisms, and peptides corresponding to each of the eight loops of P2 by immunoassay revealed that bacterial antibodies were prevalently specific for loop 5, a highly variable region, and for loop 6, a conserved surface exposed loop.

Infection of mice with *S. typhimurium* is widely accepted as a valuable experimental model for human typhoid fever. Oral infection with *S. enteric serovar Typhimurium* induces a strong T helper 1 (Th1) response that is responsible for the CD4+ T-cell-mediated protection. Cytokines such as IFN-γ and TNF-α released by Th1 cells activate bactericidal pathway in macrophages. Moreover, CD 4+ T lymphocytes help B lymphocytes to produce antibodies and salmonella-specific CD8+T lymphocytes. Various studies have demonstrated that porins induce a Th1 response (Gupta et al., 1999, M. Galdiero et al., 1998) reported that porins of *S. enteric serovar Typhimurium* elicit Th1 response in the host. In fact, porins appear to stimulate T cell proliferation in the presence of macrophages incubated with dead bacteria, live *Salmonella* infected macrophages stimulated a minor proliferation compared to dead *Salmonella* incubated macrophages. Furthermore infection with live *Salmonella* induced the loss of accessory molecules, such as B7 and ICAM-1 on macrophages. OMPA of *S. enteric serovar Typhimurium* activate dendritic cells and enhances Th1 polarization (Lee et al., 2010). Others studies demonstrate that purified porins are able to induce a different response to that induced by the porins present on the *S. typhimurium* cell surface. Porin treated or orally infected mice show anti-porin antibodies with bactericidal activity. The complete adaptive transfer of resistance to *S. typhimurium* infection is achieved only using splenic T cells from survivor mice after experimental infection. After stimulation with specific antigen in vitro CD4+ cells from porin-immunized mice released large amounts of IL-4, while CD4+ cells from *S. typhimurium* infected mice predominantly secreted IFN-γ. Limiting dilution analysis showed that infection resulted in a higher precursor frequency of IFN-γ producing CD4+ T cells and a lower precursor frequency of IL-4 producing CD4+ T cells, while immunization with porins resulted in a higher precursor frequency of IL-4 producing cells and a low frequency of IFN-γ producing cells.

Analysis of polymerase chain reaction-amplified cDNA from the spleens of infected mice demonstrated that IFN-γ, IL-2 and IL-12 mRNA were found 5 days after in vitro challenge and increased after 15 days; IL-10 expression was rarely present after both 5 and 15 days, while IL-4 mRNA expression was not detected. In porin-immunized mice, the IL-4 mRNA expression increased after 15 days, IFN-γ mRNA expression decreased after 15 days, while IL-2, IL-10 and IL-12 mRNA remained relatively unchanged (M. Galdiero et al., 1998) Other

works demonstrated that subletal doses of live *S. typhimurium* give rise to an IFN-γ dominant Th1–like immune response whereas heat-killed bacteria generate an IL-4 dominant Th2-like response (Thatte et al., 1993). Therefore, during experimental oral infection with *S. typhimurium* in mice a T-lynphocyte differentiation occurred, leading to prevalent TH1 response, while the immunization with isolated porins did not induce in vivo a similar pattern of differentiation. During the initial phase of infection with virulent strains which express large amounts of porins on their surface, *Salmonella* are present in the bloodstream and are resistant to complement-mediated lysis (Munn et al., 1982); bacterial cell is able to survive and entry into macrophages and therefore is resistant to bactericidal anti-porin antibodies. In this phase of infection, cell-mediated immunity is important for protection against typhoid fever. Transfer of immunity experiments have demonstrated that CD4+ cells, CD8+ cells and serum are all required to protect mice from challenge with virulent *S. typhimurium* (Mastroeni et al., 1993).

8. Conclusion

Porins from several Gram-negative bacteria, including Salmonella, play a fundamental role in the host-pathogen interaction, eliciting diverse biological proinflammatory activities and immune responses.

Porins present an intrinsic biological activity when interacting with eukaryotic cells, but also behave as antigens stimulating specific immune responses. Porins of *Salmonella* have endotoxin-like effects such as lethal action, the ability to elicit a local Shwartzman reaction, to activate the complement system and pyrogenicity. Furthermore, the porins stimulate pro-and anti-inflammatory cytokine synthesis and release. It has been established that protein tyrosine phosphorylation plays a central role in porin mediated transduction processes. Signal transduction pathways and transcriptional activation known to occur during immune cell activation have been widely investigated in cells stimulated by porins. Bacterial porins also may constitute important inducers of surface molecule expression on endothelial cells and contribute to endothelial transmigration of leucocytes. The protective role of porins from *Salmonella* and other bacteria has been demonstrated. Anti-porin antibodies have been shown to be bactericidal and opsonic; patients with pelvic inflammatory disease who self cure present high levels of antiporins antibodies. Porins also play an important role in the development of cellular immunity. During experimental oral infection with *S. typhimurium* in survivor mice a T-lymphocyte differentiation occurred leading to a prevalence of the Th-1 response, while the treatment with purified porins did not induce in vivo a similar pattern of differentiation. Transfer of immunity experiments have demonstrated that $CD4^+$, $CD8^+$ cells and serum are all required to protect naive mice from challenge with virulent *S. typhimurium*. These studies have led to the establishment of a multiplicity of targets for novel therapies.

9. References

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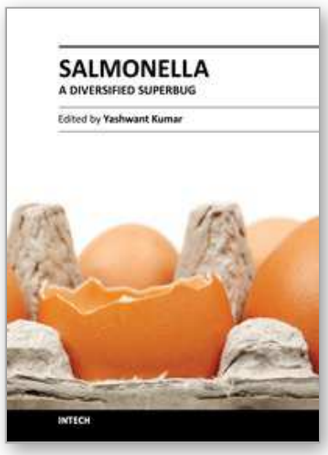

Salmonella - A Diversified Superbug
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Salmonella is an extremely diversified genus, infecting a range of hosts, and comprised of two species: enterica and bongori. This group is made up of 2579 serovars, making it versatile and fascinating for researchers drawing their attention towards different properties of this microorganism. Salmonella related diseases are a major problem in developed and developing countries resulting in economic losses, as well as problems of zoonoses and food borne illness. Moreover, the emergence of an ever increasing problem of antimicrobial resistance in salmonella makes it prudent to unveil different mechanisms involved. This book is the outcome of a collaboration between various researchers from all over the world. The recent advancements in the field of salmonella research are compiled and presented.

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