Chapter 6
Immuno-Modulatory Role of Porins: Host Immune Responses, Signaling Mechanisms and Vaccine Potential

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Introduction

Our immune system is a complex network of defense mechanisms which provides protection against a vast array of pathogens. The immune system responds to infectious microbes by triggering two branches: the innate immune system and the adaptive immune system. The innate immune system plays a crucial role during early stages of infection. Innate immune cells, like macrophages and granulocytes respond to invading pathogens by producing pro-inflammatory cytokines and chemokines leading to inflammation and killing of pathogens either directly or indirectly by activation of adaptive immune cells. The adaptive immune system combats infections effectively starting from 4 to 5 days of the infection with the help of B cell and T cell mediated responses. Sometimes, the adaptive immune system might fail to protect against the invading microbes. Some bacteria evade this defense by manipulating the immune system to establish themselves inside the human host and cause disease. At certain times, unregulated production of cytokines results in septic shock leading to multiple organ failure and ultimately death. Therefore, it is important to understand the role of pathogens in the context of host-immunomodulation.

Gram-negative pathogens pose a significant health risk to humans worldwide. The outer membrane of gram-negative bacteria contains two major components; lipopolysaccharide (LPS) and outer membrane proteins (OMPs). Among these, the bacterial endotoxin LPS is one of the well-studied immuno-stimulatory components of the bacteria and is known to cause inflammation and sepsis when present in excess.

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About one-third of the genome of gram-negative bacteria encode for OMPs (Koebnik et al. 2000). Porins are a type of OMPs that form transport channels across the membrane. They form beta barrel structures and have several roles. They act as receptors for phages and complement proteins, they can mediate antibiotic resistance (Achouak et al. 2001), anti-microbial peptide resistance (Galdiero et al. 2012), bile resistance (Wibbenmeyer et al. 2002; Hung and Mekalanos 2005) and also can act as adhesins (Duperthuy et al. 2010, 2011). All these properties suggest that they also have the ability to act as virulence factors. In fact, in *Vibrio splendidus*, one of the porins, OmpU helps in invasion of the host cells (Duperthuy et al. 2011). In *Serratia marscecens*, the opportunistic pathogen, antibiotic resistance is mainly imparted by porins (Ruiz et al. 2003). In *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*, porins trigger apoptosis of the host cells (Buommino et al. 1999; Muller et al. 2000).

Over the past two decades, accumulating evidence suggests that porins have immuno-modulatory properties. They can act as PAMPs (pathogen associated molecular patterns) and can be recognized by PRRs (pattern recognition receptors) present on host cells of mainly immune origin (Achouak et al. 2001; Galdiero et al. 2012). This review gives an overview of how porins modulate the host innate and adaptive responses, activate various signaling pathways and how they can be used as vaccines or adjuvants against various gram-negative bacterial infections.

Role of Porins in Modulation of Immune Responses

Innate Immune Responses

The innate immune system is the first line of defense against pathogen intrusion. It consists of various barriers like mechanical, chemical and physiological barriers, humoral factors and finally the inflammatory responses. The innate immune cells consist of mainly, monocytes, macrophages, dendritic cells (DCs), natural killer cells, mast cells and granulocytes such as neutrophils, basophils and eosinophils. These various cell types have specific functions that together mount an immune response towards detection and clearance of the pathogen from the host system. The innate immune responses however, do not induce memory generation and hence do not provide any additional protection upon re-challenge by the same pathogen.

Numerous gram-negative bacterial porins stimulate the production of pro-inflammatory cytokines. TNFα and IL-1β act on endothelial cells causing dilatation of vessels and hence, initiate the inflammatory process. Both these cytokines can signal the hypothalamus to induce fever. TNFα can also act on hepatocytes, along with IL-6 to induce the acute phase response. IL-12, a cytokine secreted by macrophages and dendritic cells, is involved in the differentiation of T cells. Nitric oxide is a reactive intermediate formed during phagocytosis and is toxic in nature. Porins from many gram-negative bacteria such as *Fusobacterium nucleatum*, *Haemophilus influenza*e, *Helicobater pylori*, *Neisseria*, *Salmonella* and *Shigella*

spp. induce pro-inflammatory cytokines like TNFα, IL-6 and IL-1β secretion in a variety of cell lines and primary cells, such as monocytes and macrophages of human and mouse origin (Table 6.1) (Tufano et al. 1994, 1995; Toussi et al. 2012; Galdiero et al. 2001a, b, 2004; Vitiello et al. 2004, 2011; Liu et al. 2010; Singleton et al. 2005; Massari et al. 2006; Al-Bader et al. 2004; Moreno-Eutimio et al. 2013, Galdiero et al. 2005, 2006a; Ray et al. 2003; Biswas et al. 2007; Elena et al. 2009; Pore et al. 2012). *Vibrio cholerae* OmpU stimulates monocytes and macrophages to produce TNFα and IL-6 (Sakharwade et al. 2013). Omp16 of *Brucella abortus*, an outer membrane protein lipid anchor induces TNFα and IL-12 in mouse derived macrophages (Pasquevich et al. 2010). *Pasteurella multocida* porin and *Shigella* porins induce IL-12 secretion in mouse peritoneal macrophages and HEK cells (Iovane et al. 1998; Ray et al. 2003). PorA of *Neisseria meningitidis* induces IL-12 secretion in human PBMCs (peripheral blood mononuclear cells) derived DCs (Al-Bader et al. 2004). *Salmonella* porins, OmpA of *Shigella flexineri* and OmpU of *V. cholerae* are able to stimulate nitric oxide production in macrophages of mouse origin (Sakharwade et al. 2013; Pore et al. 2012; Gupta et al. 1999). In contrast to the reports demonstrating porin-induced pro-inflammatory responses, *Salmonella* porins which are pro-inflammatory in nature can also induce the production of IL-10, a potent immune-suppressive cytokine in human cell line and mouse primary cells (Galdiero et al. 2005).

Porins can also induce secretion of chemokines such as MIP-1α, MIP-1β, RANTES and IL-8. IL-8 is a potent neutrophil chemo-attractant factor. It promotes angiogenesis and phagocytosis. Chemokines like MIP-1α and MIP1β act on granulocytes and lead to acute inflammation and increase infiltration of neutrophils at the site of infection. They also aid in release of pro-inflammatory cytokines from macrophages. RANTES recruits T cells, eosinophils, basophils and leukocytes to inflammatory sites. OmpU deleted strain of *V. cholerae* showed decreased production of pro-inflammatory cytokines along with chemokines (Bandyopadhaya et al. 2007b, 2009; Sarkar et al. 2012). PorA of *N. meningitidis* and *Shigella* porins induce strong chemokine response in human PBMCs derived DCs and mouse peritoneal macrophages respectively (Al-Bader et al. 2004; Ray et al. 2003; Biswas et al. 2007).

Further, several studies on how porins affect neutrophil functions have been carried out. *Nesserial* porins are able to inhibit chemokine induced actin polymerization as well as degranulation in human PBMCs derived neutrophils (Bjerknes et al. 1995). Further, meningococcal porins down-regulate complement receptors CD35 and CD11b on neutrophils, but increase their oxidative burst capacity (Bjerknes et al. 1995). However, PorB of *N. gonorrhoeae* down-regulates oxidative burst and inhibits granule fusion with plasma and phagosomal membranes (Lorenzen et al. 2000). *Salmonella* Typhimurium porins induce the production of platelet-activating factor by human neutrophils (Tufano et al. 1992) as well as cause leukocyte transmigration *in vitro* (Galdiero et al. 1999). *Pasteurella haemolytica* porins decrease phagocytic index and intracellular killing capacity of bovine polymorphonuclear leukocytes (Galdiero et al. 1998b). *Klebsiella pneumoniae* OmpK35 and OmpK36 may affect neutrophil phagocytosis as deletion mutants caused an

Table 6.1 Innate immune responses initiated by porins

Organism	Porin	Model	Signaling mediators implicated	Inflammatory response	References
Brucella abortus	Omp16	Mouse macrophages		TNFα, IL-12	Pasquevich et al. (2010)
Fusobacterium nucleatum	FomA	HEK	TLR2 dependent NFκB activation	IL-8	Toussi et al. (2012)
Haemophilus influenza	Hib	Mice	TLR2-MyD88 expression along with CD14	TNFα, IL-6	Galdiero et al. (2004)
		THP-1		TNFα, IL-6	
		HEK		IL-8	
	P2	U87-MG		IL-6	Vitiello et al. (2011)
		U937	MEK1-MEK2-MAPK pathway		Galdiero et al. (2003c)
	Hib	Rat brain		TNFα, IL-1α, MIP-2	Galdiero et al. (2001a)
Helicobacter pylori	30 kDa porin	Human PBMCs derived monocytes		TNFα, IL-6, IL-8	Tufano et al. (1994)
Klebsiella pneumoniae	35 and 36 kDa	Human PMNs		Induction of opsonizing antibodies	Alcantar-Curiel et al. (2000)
Neiserria lactamica	PorB	BEAS-2B		IL-8	Liu et al. (2010)
		Detroit 562 cells			
Neiserria meningitidis	PorB	HEK 293	TLR2/TLR1 mediated NFκB activation	IL-8	Massari et al. (2006)
		Mouse splenic dendritic cells		IL-6	Singleton et al. (2005)
	PorA	Human monocyte derived dendritic cells		IL-8, RANTES, MIP-1α, MIP-1β,	Al-Bader et al. (2004)
				TNFα, IL-6, IL-12p40	
Pasteurella haemolytica	LAP	Calves and sheep lungs		Acute bronchopneumonia	Brogden et al. (1995)

Pasteurella multocida	37.5 kDa porin	Murine splenocytes		IL-1α, IL-6, TNF-α, IFN-γ	Iovane et al. (1998)
				IL-1α, IL-6, TNF-α, IFN-γ, IL-12p40	
Pseudomonas aeruginosa	38 kDa	Mice		Fibroblast increase; collagen edema	Baroni et al. (2001)
	38 kDa	Human PBMCs		TNFα	Cusumano et al. (1997)
				IL-6	
***Salmonella* Typhi**	OmpS1	Mouse bone marrow derived macrophages		TNFα, IL-6, IL-10	Moreno-Eutimio et al. (2013)
				Increased CD40 expression by OmpS2	
	OmpS2	Mouse spleen and lymph node			
***Salmonella* Typhimurium**	34 and 36 kDa porins	Mouse		TNFα, IL-6	Tufano et al. (1995)
		RAW 264.7	p38 and JNK MAPK activation	Nitric oxide	Vitiello et al. (2008a)
		Mouse gut macrophages		Nitric oxide	Gupta et al. (1999)
		U937 monocytes	Raf-1-MEK1-MEK2-MAPK pathway leading to activation of NFκB and AP-1 activation	TNF-a, IL-1β, IL-6, IL-10 and IL-1β	Galdiero et al. (2002, 2003a, 2005, 2006a), Finamore et al. (2009)
			PTK induced PKA and PKC activation		
		THP-1	PTK and p38 MAPK leading to activation of NFκB and AP-1 activation	TNFα, IL-6, IL-8	Vitiello et al. (2004), Galdiero et al. (2001b)

(continued)

Table 6.1 (continued)

Organism	Porin	Model	Signaling mediators implicated	Inflammatory response	References
Shigella dysenteriae	MOMP	Mouse peritoneal macrophages	TLR2/6 mediated MyD88 dependent NFκB activation	TNFα, IL-12	Ray et al. (2003), Biswas et al. (2007)
				MIP-1α, MIP-1β, RANTES	
				Increase in CD80, MHC-II, CD40 expression	
		Int407	TLR2/6 mediated MyD88 dependent NFκB activation	MIP-1α, MCP-1, IL-8	Mukherjee et al. (2014)
Shigella flexneri	38 and 40 kDa	Caco-2	Up-regulation of TLR2 and MyD88	TNFα, IL-8, IL-1β	Elena et al. (2009)
	38 kDa	Mouse peritoneal macrophages		MIP-1α, MIP-1β and RANTES	Biswas et al. (2007)
				TNFα, IL-12	Biswas et al. (2007), Ray et al. (2003)
	OmpA	HEK 293		IL-6, IL-12p70, and IL-1β	Pore et al. (2012)
		Mouse peritoneal macrophages		Nitric oxide	
				Nitric oxide	
		RAW 264.7		Increase in CD80 and MHC-II expression	
Vibrio cholerae	OmpU	RAW 264.7		Nitric oxide, TNFα, IL-6	Sakharwade et al. (2013)
		THP-1, human PBMCs		TNFα, IL-6	
	OmpU deleted strain	Int407		IL-1α, IL-6, MCP-1	Bandyopadhaya et al. (2007a)
				TNF-a, IL-6, IL-1α	Bandyopadhaya et al. (2009)
				IL-8	Sarkar et al. (2012)

Cell lines: BEAS-2B (human airway epithelial cell line), Caco-2 (human epithelial colorectal adenocarcinoma cell line), Detroit 562 cells (human pharynx carcinoma cell line), HEK (human epithelial kidney cell line), Int407 (human intestinal epithelial cell line), RAW 264.7 (murine macrophage cell line), THP-1 (human monocytic cell line), U87-MG (human astroglioma cells), U937 (human leukemic monocytic cell line)

increase in phagocytosis capacity (Tsai et al. 2011). *H. pylori* porins decrease chemotaxis ability of human neutrophils and can interfere with intracellular killing (Tufano et al. 1994).

The complement system falls under the humoral branch of the innate immune system. This system consists of plasma proteins that interact with each other and ultimately lead to opsonization of pathogens or induction of several inflammatory responses. There are three pathways (classical, mannan binding lectin and alternative pathways) by which the complement system is activated which subsequently converge at C3 convertase enzyme and formation of membrane attack complex (MAC) that leads to killing of target microbes. C3b can opsonize microbes by binding to complement receptors on phagocytes. C3a, C4a and C5a can recruit phagocytes to inflammatory sites. Most porins activate the classical pathway by binding to C1q.

Porins from *Salmonella minnesota* bind to C1q (Latsch et al. 1990). *N. gonorrhoeae* Por1B binds to C3b and C4 (Lewis et al. 2008) and Por1A and Por1B bind to C4 binding protein as well (Ram et al. 2001). Similarly, *N. meningitidis* OMPs activate the complement system (Bjerre et al. 2002). *S.* Typhimurium porins activate the classical complement pathway as measured by consumption of C1s, C3 and C4 in human or guinea pig serum (Galdiero et al. 1984). *K. pneumoniae* OmpK36 also activates the classical complement pathway *in vivo* by binding to C1q and leads to deposition of C3, C5-9 (MAC) components on the porin (Alberti et al. 1993, 1996). Similarly, *Aeromonas hydrophila* 39 kDa porin and *Aeromonas salmonicida* 40 kDa porin activate the classical pathway in an antibody independent manner by binding to C1q (Merino et al. 1998, 2005). The MOMP (major outer membrane protein) of *Legionella pneumophila* binds to C3 and cause phagocytosis of MOMP incorporated vesicles by human monocytes (Bellinger-Kawahara and Horwitz 1990).

All these facts have led to the understanding that porins are able to induce pro-inflammatory cytokine and chemokine responses as studied *in vitro* and *in vivo* in both mouse and human cells. Some porins also have the ability to induce production of cytokines involved in activation of cells important for innate immune responses or adaptive immune responses. Further, porins are able to interfere with neutrophil function as well as activate the complement system. Interestingly, observation from our laboratory revealed that *V. cholerae* OmpU is able to down-regulate LPS mediated effects, although it is pro-inflammatory in nature.

Adaptive Immune Response

The adaptive response starts later as the infection progresses; it is specific, more potent than the innate immune responses and is associated with memory induction. The adaptive immune response is initiated with the help of signals generated by the innate immune cells which can activate lymphocytes; the T and B cells. Antigen

presenting cells such as, macrophages and dendritic cells, present endocytosed antigens to $CD4^+$ and $CD8^+$ T cells and activate them. Activated T helper cells (Th) further help in B cell mediated antibody responses. T cells require certain signals from antigen presenting cells (APCs) in order to be activated optimally. The interaction of antigen presented by MHC molecules on APCs and certain co-stimulatory molecules like B7 (CD80 and CD86), CD40 etc. with their respective receptors present on T cells, trigger their activation. Cytokines also play an important part in differentiation of T cells. In presence of cytokines such as IL-12 and IFNγ, $CD4^+$ T cells differentiate towards Th1 type and in the presence of IL-4, $CD4^+$ T cells differentiate towards Th2 type. Th1 cells activate macrophages and differentiation of B cells, followed by antibody production, shaping the immune responses towards cell-mediated immunity (Fig. 6.1). On the other hand, Th2 polarization is required for humoral immunity and hyper-sensitivity.

Some porins can modulate adaptive-responses of the host. Porins of *Shigella*, *Salmonella* and *Neisseria* species affect antigen presenting cells such as macrophages and dendritic cells in numerous ways. *Shigella* porins induce expression of CD40 and CD80 co-stimulatory molecules as well as MHC-II molecules on macrophages of mouse origin (Elena et al. 2009; Pore et al. 2012). *Salmonella* porins induce expression of CD40 and CD86 on dendritic cells of mouse origin (Moreno-Eutimio et al. 2013; Cervantes-Barragan et al. 2009). *N. meningitidis* PorA and PorB increase expression of co-stimulatory molecules along with MHC-II molecules in human PBMCs derived DCs and mouse splenic DCs respectively (Al-Bader et al. 2004; Singleton et al. 2005). OmpA porin of *Acinetobacter baumannii* can stimulate mouse bone marrow derived dendritic cells to secrete IL-12 along with

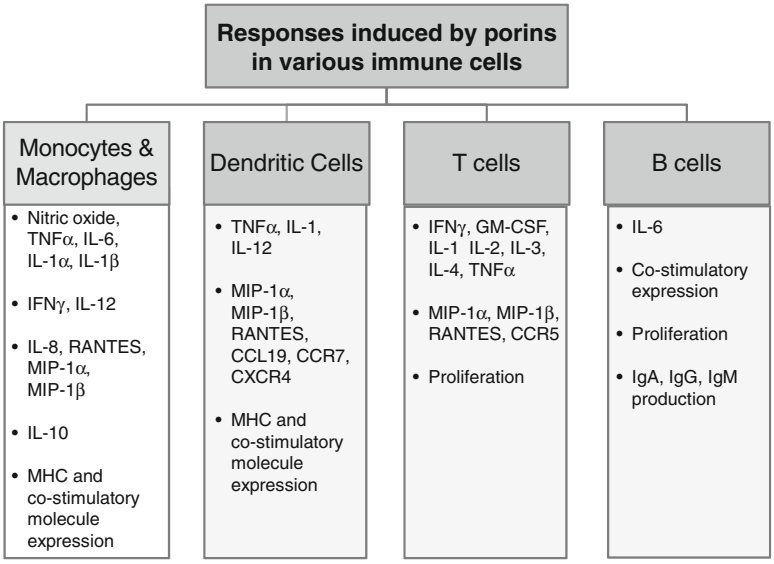

Fig. 6.1 Different immune responses elicited by gram negative porins in different types of immune cells

increased surface expression of co-stimulatory moleculesas well as maturation of dendritic cells and can polarizes T cells towards Th1 type response (Lee et al. 2007). *S*. Typhimurium porins induce Th1 and Th2 differentiation of T cells (Galdiero et al. 1998a). Many *S*. Typhimurium porins have been studied for the effect on B cell responses. Collectively, these porins can induce co-stimulatory molecule expression of B cells of mouse and human origin (Cervantes-Barragan et al. 2009; Galdiero et al. 2003b). Also, they generate IgM and IgG antibody responses (Secundino et al. 2006; Gil-Cruz et al. 2009). Similarly, porins of *N. meningitidis* and *N. gonorrheae* cause generation of IgM responses and induce CD86 expression in mouse splenic B cells (Snapper et al. 1997; Wetzler et al. 1996). *S. dysenteriae* MOMP can induce co-stimulatory molecule expression in mouse peritoneal B1 and B2 cells and also generate IgM, IgA and IgG responses (Ray et al. 2004; Ray and Biswas 2005). The *Helicobacter pylori* 30 kDa porin induces IFNγ, GM-CSF, IL-3 and IL-4 secretion in lymphocytes derived from human (Tufano et al. 1994) which increase proliferation of mast cells, decrease IFNγ secretion by macrophages, induce class switching in B cell and differentiation of Th2 cells.

So far, the literature indicates that various gram-negative porins have the capacity to induce adaptive immune responses. Porins can provide signal for Th1 or Th2 differentiation as well as B cell activation, class switching phenomenon and affinity maturation.

Signaling Cascades Initiated by Porins

Identification of pathogenic and non-pathogenic organisms by innate immune cells occurs upon recognition of various PAMPs by PRRs (Medzhitov and Janeway 1997; Kumar et al. 2012; Kawai and Akira 2009). PRRs, then initiate intracellular signaling pathways that lead to recruitment of phagocytic cells, monocytes to the site of infection and activation of innate and adaptive immunity (Medzhitov 2007).

TLRs are one of the major type of PRRs present on immune cells (Akira and Takeda 2004; Kaisho and Akira 2001; Armant and Fenton 2002). Upon binding to specific microbial components, TLRs trigger intracellular signaling cascades that result in production of inflammatory cytokines and chemokines from several immune cells (Akira and Takeda 2004; West et al. 2006; Mogensen 2009) (Fig. 6.2). These inflammatory cytokines can induce dendritic cell maturation which is characterized by up-regulation of co-stimulatory molecules and altered expression of chemokine receptors on the surface of DCs. Thus, TLR mediated DC maturation acts a link between innate and adaptive immunity (Akira et al. 2001).

TLR signaling further activates transcription factors such as, NFκB and AP-1 (Bell et al. 2003; Kawai and Akira 2005; Karin and Greten 2005). Briefly, TLRs upon binding to specific ligands interact with intracellular TIR domain containing adaptor molecule MyD88. MyD88 then recruits IRAK1 (IL-1 receptor associated kinase 1) which then forms a complex with IRAK4 or IRAK2. Phosphorylated IRAK1 recruits TNF receptor associated factor 6 (TRAF6) and E2 ubiquitin conjugating enzyme 13 (UBC13). TRAF6 and UBC13 poly-ubiquitinylate IRAK1 and TRAF6, leading to activation of MAPK and NFκB pathways.

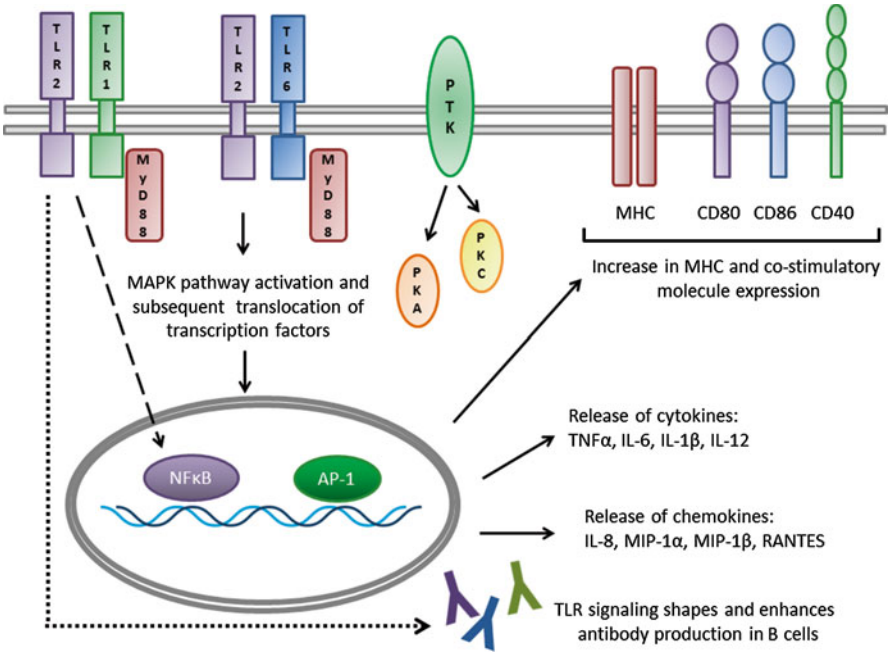

Fig. 6.2 Gram negative porins can stimulate TLR pathway and result into cell surface expression of molecules of immune importance and release of pro-inflammatory cytokines, chemokines and antibody secretion by B cells

Several studies suggest that porins act as PAMPs as they interact with TLRs and initiate down-stream signaling. Porins of various gram-negative bacteria mediate signaling via TLR pathway (Table 6.1). Data from the studies on *Shigella*, *Neisseria* and *Heamophilus* spp. indicate that these porins are recognized by TLR2 predominantly heterodimerized with either TLR1 or TLR6. Upon binding to porins, TLR2/TLR1 or TLR2/TLR6 activate NFκB or AP-1 via MyD88 dependent pathway leading to translocation of the nuclear factors into the nucleus and transcription of pro-inflammatory cytokine genes and chemokine genes mediated by various cytosolic signaling cascades (Massari et al. 2002, 2006; Banerjee et al. 2008; Ray and Biswas 2005; Biswas et al. 2007; Singleton et al. 2005). *S.* Typhimurium porins induce phosphorylation of protein tyrosine kinases (PTK), protein kinase A (PKA) and protein kinase C (PKC) in U937 monocytic cell line and also activate transcription factors AP-1 and NFκB by Raf-1-MEK1/2-MAPK pathway (Galdiero et al. 2002, 2003a). Inhibitor studies suggest that p38 MAPK is mainly involved in transcription factor activation. Studies on *Neisseria* spp. porins are implicated in TLR mediated NFκB activation which occurs via Raf-1-MEK1/2-MAPK pathway; (Massari et al. 2003; MacLeod et al. 2008). *H. influenzae* porin P2 and porins of *Salmonella* and *Neisseria* also activate the MAPK pathway (Galdiero et al. 2002, 2003c; Vitiello et al. 2004; MacLeod et al. 2008). The three-dimensional structure model of porin P2 constructed on the basis of crystal structure of *K. pneumoniae* OmpK36 and *Escherichia coli* PhoE and OmpF predict that the domains of surface exposed loops are involved in activation of signal transduction pathway (Table 6.1). In particular,

Table 6.2 Adaptive responses initiated by porins

Cell type	Organism	Porin	Model	Effect on adaptive responses	References
Dendritic cells	*Acinetobacter baumannii*	OmpA	Bone marrow	Increase in CD40, CD54, CD80 and CD86	Lee et al. (2007)
				Production of IL-12	
				Augmentation of syngeneic and allogeneic immunostimulatory capacity	
	Neiserria meningitidis	PorA	Human monocyte derived dendritic cells	Increase in expression of MHC-II, CD40, CD54, CD80, CD86	Al-Bader et al. (2004)
				Decrease in receptor mediated endocytosis	
				Induction of allostimulatory activity	
				Induction of $CD4^+$ T cell proliferation	
		PorB	Mouse spleen	Increase in CD86 and MHC molecules	Singleton et al. (2005)
				Induction of allostimulatory activity	
				Activation of antigen specific T cells	
	Salmonella Typhi	OmpS1, OmpS2	Mouse bone marrow derived DCs	TNFα, IL-6, IL-10 expression	Moreno-Eutimio et al. (2013)
			Mouse spleen and lymph nodes	Increased in MHC-II and CD40 expression	
	Salmonella Typhimurium	OmpC and OmpF	Mouse spleen	Increase in CD86 and CD40 expression	Cervantes-Barragan et al. (2009)
	Shigella dysenteriae	MOMP incorporated liposome	Mouse spleen	Up-regulation of MHC class II, CD40, CD80 and ICAM-1	Banerjee et al. (2008)
				Increase in expression of TNFα, IL-12, MIP-1α, MIP-1β, RANTES, CCL19, CCR7, CXCR4	

(continued)

Table 6.2 (continued)

Cell type	Organism	Porin	Model	Effect on adaptive responses	References
T cells	*Acinetobacter baumannii*	OmpA	Mouse spleen	Induction of IFNγ	Lee et al. (2007)
	Helicobacter pylori	30 kDa porin	Human PBMCs derived lymphocytes	IFNγ, GM-CSF, IL-3, IL-4	Tufano et al. (1994)
	Salmonella Typhimurium	34 and 36 kDa	Mouse spleen	IFNγ and IL-4 release by $CD4^+$ T cells purified from immunized mice	Galdiero et al. (1998a)
	Shigella dysenteriae	MOMP	Mouse spleen	Induction of IL-2, CD25, MIP-1α, MIP-1β, RANTES, CCR5	Biswas et al. (2008, 2009)
				Release of TNFα, IFNγ	
	Shigella flexneri	OmpA	Mouse spleen	Release of IL-2, IFNγ	Pore et al. (2012)
				Induction of MIP-1α, MIP-1β, RANTES, CCR5	

B cells	*Fusobacterium nucleatum*	FomA	Mouse spleen	IL-6 production	Toussi et al. (2012)
				Increase in CD86 and MHC-II expression	
	Neiserria gonorrheae	PIA, PIB	Mouse spleen and lymph node	Increase in CD86 expression	Wetzler et al. (1996)
			Mouse spleen	Increase in IgM secretion	Snapper et al. (1997)
		PorB		Increase in B cell proliferation	Wetzler et al. (1996)
				Increase in T cell co-stimulatory activity	
	Neiserria meningitidis	C1, C3	Mouse spleen and lymph node	Increase in CD86 expression	Wetzler et al. (1996)
		C3	Mouse spleen	Increase in IgM secretion	Snapper et al. (1997)
		PorB	Mouse spleen	Increase in CD86 expression	MacLeod et al. (2008)
				Increase in proliferation	
				PTK-MAPK pathway involved	
	Salmonella Typhimurium	OmpD	Mouse spleen	Increase in IgM secretion	Gil-Cruz et al. (2009)
				Induction of B cells showing B1b markers	
		OmpC	Mouse spleen	Increase in IgG2b, IgG1, IgG2a production	Secundino et al. (2006)
		OmpC and OmpF	Mouse spleen	Increase in MHC-II, CD86 and CD40 expression	Cervantes-Barragan et al. (2009)
		34 and 36 kDa	Human PBMCs	Increase in CD80 and CD86 expression	Galdiero et al. (2003b)
	Shigella dysenteriae	MOMP	Mouse peritoneal cavity B2 cells	Increase in of expression of CD86, IgA, IgM, IgG2a	Ray and Biswas (2005)
			Mouse peritoneal B1 cells	Up-regulation of CD80–CD86, IgA, IgM	Ray et al. (2004)
				TLR2/6-MyD88 involvement	

synthetic peptide corresponding to surface exposed loops L5, L6 and L7 activate JNK and p38 MAPK similarly as the intact protein with L7 being the most active peptide (Galdiero et al. 2003c). Further studies on L7 showed that only six amino acids contribute to the overall activity and induction of TNFα and IL-6 production (Galdiero et al. 2006b).

Porins as Vaccine Candidates

For an agent to be used as a good vaccine, it must be highly immunogenic and a major protective antigen. It is desirable for a vaccine candidate to drive the $CD4^+$ T cell responses towards Th1 to ensure both humoral and cell mediated immunity against pathogens. Activated Th1 cells aid in reduction and clearance of pathogens (intra-cellular and extra-cellular) by secreting IFN-γ, TNFα, IL-2 and IL-3 and help in activation and differentiation of B cells, $CD8^+$ T cells and macrophages. In certain cases, like anti-parasite responses, Th2 differentiation is important. Th2 cells produce IL-4, IL-5, IL-13, IL-6 and IL-10 and mainly support B cell activation and differentiation. $CD8^+$ T cells clear intra-cellular pathogens by killing infected cells or by releasing cytokines that would help in the process. Antibodies produced by B cells can bind to the enzymatic active sites of toxins or prevent their diffusion, neutralize viral replication, promote phagocytosis of extracellular bacteria by opsonization and can activate the complement cascade. IgM followed by IgG antibodies appear a few days after immunization. B cell maturation is associated with two major events: Ig class-switch recombination from IgM towards IgG, IgA or IgE, and maturation of the affinity of B cells for their specific antigen.

Porins have been widely studied for their capacity to act as adjuvants or as potential vaccines in various animal models (Table 6.3). *A. hydrophila* is a gram-negative organism that is pathogenic in fish, amphibian and humans as well. Administration of OmpF of *A. hydrophila* leads to increased IgG expression in mouse model along with increased lymphocyte proliferation and T cell activation *in vitro* (Yadav et al. 2014). OmpTS of *A. hydrophila* was highly immunogenic in fish model *Labeo rohita* and Omp48 immunized fish showed survival against *A. hydrophila* and *Edwardsiella tarda* infections (Khushiramani et al. 2007, 2014). *Borrelia burgdorferi* is a spirochete that causes Lyme's disease. One of its porins, Oms66 showed protection against infection in immunized mice (Exner et al. 2000). Mice immunized with Omp16 from *B. abortus*, that causes brucellosis, showed protection from infection. Further, Omp16 was able to activate dendritic cells and induce IFNγ secretion from mouse splenic T cells and induce foot pad swelling (Pasquevich et al. 2010). *Burkholderia pseudomallei* infects both animals and humans causing melioidosis, which has a mortality rate of 20–50 % in humans even with treatment. Mice immunized with OmpA of *B. pseudomallei* showed protection against infection (Hara et al. 2009). *Chlamydia trachomatis* is an obligate intracellular pathogen that causes urethritis, proctitis, trachoma, infertility and is the single most infectious agent associated with blindness. Administration of *C. trachomatis* MOMP in mice

Table 6.3 Porins and outer membrane proteins as vaccine candidates or adjuvants

Organism	Porin	Administered as (route)	Model	Effect observed	References
Acinetobacter baumannii	Outer membrane vesicles	Intra-muscular	Mouse	Protection against two *A. baumannii* strains	McConnell et al. (2011)
		With alum as adjuvant			
Aeromonas hydrophila	OmpF	Intra-peritoneal	Mouse	Increased IgG expression	Yadav et al. (2014)
		With Freund's adjuvant		Increased lymphocyte proliferation *in vitro*	
				IL-4 and IFNγ secretion by T cells *in vitro*	
	Omp48	Intra-muscular	*Labeo rohita*	Immunized fish showed increased survival against *A. hydrophila* and *E. tarda*	Khushiramani et al. (2014)
	OmpTS	Intra-peritoneal	*Labeo rohita*	Highly immunogenic in fish	Khushiramani et al. (2007)
		With Freund's adjuvant			
Borrelia burgdorferi	Oms66	Skin implantation	Mouse	Protection against infection	Exner et al. (2000)
Brucella abortus	Omp16	Intra-peritoneal and oral	Mouse	Induction of IFNγ by splenic T cells	Pasquevich et al. (2010)
		Oral		Foot pad swelling	
		Intra-venous		Activation of dendritic cells	
		Plant derived Omp16; Oral		Protection against infection	
Burkholderia pseudomallei	OmpA	Intra-peritoneal	Mouse	Protection against *B. pseudomallei* infection	Hara et al. (2009)

(continued)

Table 6.3 (continued)

Organism	Porin	Administered as (route)	Model	Effect observed	References
Chlamydia trachomatis	MOMP	Intra-muscular MOMP DNA priming with ISCOM	Mouse	Stronger delayed-type hypersensitivity	Dong-Ji et al. (2000)
				IFNγ and IgA production	
				Protection against lung challenge	
		Sub-cutaneous administration of sonicated and vortexed MOMP with Freund's adjuvant	Mouse	Increased IgG and IgA production by sonicated MOMP	Pal et al. (2001)
				Increased T cell responses (proliferation, IL-4 and IFNγ production) by vortexed MOMP	
				Sonicated MOMP immunization showed protective effect upon genital challenge	
		Intra-venous adoptive immunization with antigen-pulsed DCs	Mouse	Proliferation of infection-sensitized $CD4^+$ T	Shaw et al. (2002)
				IL-12 and IFNγ secretion	
				No protection against infection	
		Intra-venous adoptive transfer of adenoviral transfected DC	Mouse	Increased expression of MHC-II, CD80 on DC	Lu et al. (2010)
				Increased IL-12 secretion	
				Protection against genital tract challenge infection	
	MOMP	Intra-muscular	*Macaca mulatta*	High amount of IgG and IgA present in plasma, vaginal washes, tears, saliva, and stool	Cheng et al. (2011)
	Subunit vaccine	With CpG-2395 and Montanide ISA 720 VG as adjuvants		Lymphoproliferative response	
				TNFα, IFNγ production	
	Porin B and Porin D in *V. cholerae* ghosts	Intra-muscular	Mouse	Cross protection against *C. muridarum*, *C. trachomatis* and *V. cholerae*	Eko et al. (2011)
	Multi subunit vaccine			Cross-reactive chlamydial specific genital mucosal Th1/Th2 cytokine responses and IgA and IgG2a antibody responses	

Fusobacterium nucleatum	FomA	Subcutaneous With OVA/alum as adjuvant	Mouse	Increased IgG and IgM production IL-10 and IL-6 expression	Toussi et al. (2012)
Neiserria lactamica	PorB	Sub-cutaneous With OVA as adjuvant	Mouse	High titers of IgG1 and IgG2b Production of IL-4, IL-10, IL-12 and INF-γ	Liu et al. (2008)
Neisseria gonorrhoeae	Class I	Nasal	Mouse	Clearance of vaginal infection	Plante et al. (2000)
	PorB DNA vaccine	Intra-muscular Epidermal particle bombardment	Mouse	Th1 responses Th2 responses	Zhu et al. (2004)
Neisseria meningitidis	PorA in liposomes	Sub-cutaneous With alum as adjuvant	Mouse	Anti-sera showed bactericidal activity	Christodoulides et al. (1998)
		Intra-peritoneal	Mouse	Anti-sera showed bactericidal activity	Humphries et al. (2004)
	PorB	Intra-peritoneal With alum as adjuvant	Mouse	Anti-sera showed bactericidal activity	Wright et al. (2002)
		Intra-nasal	Mouse	Protective effect as adjuvant against *Francisella tularensis* infection	Chiavolini et al. (2008)
Pseudomonas aeruginosa	OmpF epitopes expressed in CPMV	Sub-cutaneous With Freund's and QuilA adjuvants	Mouse	IgG2a response	Brennan et al. (1999)
	Recombinant OprF and OmpI fusion proteins	Intra-peritoneal With alum as adjuvant	Mouse	Protection upon challenge with *P. aeruginosa*	von Specht et al. (1995)
	OprF Expressed in plasmid vector	Intra-dermal (Gene gun)	Mouse	IgG1 response Protection to pulmonary infection by *P. aeruginosa*	Price et al. (2001)

(continued)

Table 6.3 (continued)

Organism	Porin	Administered as (route)	Model	Effect observed	References
***Salmonella* Typhi**	OmpC, OmpF and OmpA	Intra-peritoneal	Mouse	Administration of two or three porins retard lethal effect; None were protective on their own	Toobak et al. (2013)
		With Freund's adjuvant			
	OmpS1, OmpS2	Intra-peritoneal	Mouse	Protection against *S*. Typhi infection	Moreno-Eutimio et al. (2013)
		With OVA adjuvant		Act as adjuvants	
	34, 36 kDa	Intra-peritoneal	Mouse	Protection against *S*. Typhi infection	Isibasi et al. (1992)
	34, 35, 36 kDa	Intra-peritoneal	Mouse	Protection against *S*. Typhi infection	Singh et al. (1999)
	OmpA	Intra-peritoneal	Mouse	Protection against *S*. Typhi infection	Isibasi et al. (1988)
***Salmonella* Typhimurium**	34, 35, 36 kDa	Sub-cutaneous	Mouse	Protection against *S*. Typhimurium and *S. enterditis*	Tabaraie et al. (1994)
	LT2	Intra-peritoneal	Mouse	Protection against *S*. Typhimurium challenge	Matsui and Arai (1990)
Treponema pallidum	Outer membrane vesicle	Sub-cutaneous	Mouse	Anti-OMV serum possessed complement-dependent treponemicidal activity	Blanco et al. (1999)
				Antibody response against TROMPs	
***Vibrio* strains**	OmpK	Intra-peritoneal	*Epinephelus coioides*	Tolerance to infection by pathogenic strains	Li et al. (2010b)
***V. harveyi* (11)**		With Freund's incomplete adjuvant			
***V. alginolyticus* (6)**					
***V. parahaemolyticus* (2)**					

Vibrio anguillarum	Omp38 DNA construct	Oral	*Lates calcarifer*	Moderate protection against *V. anguillarum* infection	Rajesh Kumar et al. (2008)
Vibrio alginolyticus	OmpW	Intra-peritoneal	*Larimichthys crocea*	Resistance against *V. alginolyticus* infection	Qian et al. (2007)
	OmpU	Intra-peritoneal	*Lutjanus erythropterus*	Protection against *V. alginolyticus* infection	Cai et al. (2013)
Vibrio cholerae	22, 30, 42, 43 kDa OMPs anti-sera	Ileal loop challenge	Rabbit	Reduced *V. cholerae*-induced fluids secretion in ileal loop	Das et al. (1998)
	Outer membrane vesicle	Intra-peritoneal	Mouse	Protection against *V. cholerae* infection	Schild et al. (2008); Leitner et al. (2013)
Vibrio harveyi	VhhP2	Oral and intra-peritoneal	*Paralichthys olivaceus*	Immunoprotection against *V. harveyi* infection	Sun et al. (2009)
	OmpK	Intra-peritoneal	*Epinephelus coioides*	Protection against *V. harveyi* infection	Ningqiu et al. (2008)
		With Freund's incomplete adjuvant			
		Intra-peritoneal	*Pseudosciaena crocea*	Protection against *V. harveyi* infection	Zhang et al. (2007)
Vibrio parahaemolyticus	VP1061, VP2850	Intra-peritoneal	*Carassius carassius*	Cross-protective immune reaction against the infections of *V. alginolyticus*, *A. hydrophila*, and *P. fluorescens*	Li et al. (2010a)
		With Freund's incomplete adjuvant	Mouse		
	OmpW, OmpV, OmpU and OmpK	Intra-peritoneal	*Larimichthys crocea*	Protection against *V. parahaemolyticus* infection	Mao et al. (2007)

induced IgG, IgM responses; T cells responses, co-stimulatory molecule expression in dendritic cells along with IL-12 secretion (Dong-Ji et al. 2000; Pal et al. 2001; Shaw et al. 2002). MOMP immunized mice showed protection against genital challenge by the bacteria (Lu et al. 2010). MOMP subunit vaccine administered in rhesus macaques also showed similar T and B cell responses (Cheng et al. 2011). Other porins of *C. trachomatis*, Porin B and Porin D, when administered to mice in the form of *V. cholerae* ghosts, induced cross-reactive chlamydial specific genital mucosal T and B cell responses (Eko et al. 2011). FomA of *F. nulceatum*, bacteria involved in periodontal disease, leads to IgG and IgM antibody production along with IL-6 and IL-10 secretion upon treatment in mice (Toussi et al. 2012). *Neiserria lactamica* is a commensal found in infants that can cause pneumonia in children. PorB induces high levels of IgA and IgM antibody responses along with IL-4, IL-12, IL-10 and IFNγ production in mice (Liu et al. 2008). Similarly, PorB of *N. gonorrhoeae*, the bacteria that causes gonorrhea, induces Th1 and Th2 type responses in mice (Zhu et al. 2004). Mice immunized with Class I porins of *N. gonorrhoeae*, showed reduction in vaginal infection (Plante et al. 2000). PorB of *N. meningitidis*, the causal bacteria of meningococcal disease, showed a protective response against *Francisella tularensis* infection (Chiavolini et al. 2008). Further, antisera of *N. meningitidis* PorB immunized mice showed bactericidal activity (Wright et al. 2002). Similarly, antisera of *N. meningitidis* PorA incorporated liposome immunized mice showed bactericidal activity (Christodoulides et al. 1998; Humphries et al. 2004). *P. aeruginosa* is an opportunistic pathogen that colonizes in the lungs, kidneys and urinary tract. *P. aeruginosa* OmpF epitopes induced IgG2a response in mice whereas OprF induced IgG1 response in mice (Brennan et al. 1999). Mice immunized with OprF and OmpI fusion proteins or OprF only were able to resist infection (von Specht et al. 1995; Price et al. 2001). *Salmonella* causes food poisoning that is characterized by enteritis and diarrhea, leading to typhoid. Administration of various *Salmonella* Typhi porins offer protection against infection in mice. OmpA, OmpC, OmpF, OmpS1 and OmpS2 have been studied in this regard (Toobak et al. 2013; Moreno-Eutimio et al. 2013; Isibasi et al. 1988, 1992; Singh et al. 1999). OmpS1 and OmpS2 also show adjuvant properties (Moreno-Eutimio et al. 2013). Various *S*. Typhimurium porins immunized mice have also shown protection to infection (Tabaraie et al. 1994; Matsui and Arai 1990). Outer membrane vesicles (OMVs) of *Treponema pallidum*, the causative agent of syphilis, when administered to mice showed an antibody response against outer membrane proteins. Anti-OMV serum showed complement dependent treponemicidal activity (Blanco et al. 1999). OMVs of *A. baumanii* immunized mice showed protection against two strains of *A. baumanii* (McConnell et al. 2011). Similarly, administration of *V. cholerae* OMVs showed protection against cholera in mice (Schild et al. 2008; Leitner et al. 2013). Anti-sera against 22, 30, 42 and 43 kDa *V. cholerae* OMPs reduced *V. cholerae* induced fluid secretion in ileal loop model in rabbits (Das et al. 1998). Many *Vibrio spp*. like *V. anguillarum*, *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus* affect fish and other marine animals. Sea food contaminated with *V. parahaemolyticus* can cause gastroenteritis in humans. *V. anguillarum* Omp38 immunized Asian seabass showed moderate protection against infection

(Rajesh Kumar et al. 2008). Similarly, immunization with OmpW and OmpU of *V. alginolyticus* showed protection upon challenge with the bacteria in large yellow croaker and crimson snapper respectively (Qian et al. 2007; Cai et al. 2013). Immunization of fish with certain *V. harveyi* outer membrane proteins was able to protect fish from infection. Vhhp2 administration in olive flounder and OmpK immunization of large yellow croaker and orange spotted grouper had a protective effect against *V. harveyi* infection in the fishes (Sun et al. 2009; Ningqiu et al. 2008; Zhang et al. 2007). Large yellow croaker fish immunized with *V. parahaemolyticus* OmpW, OmpV, OmpU and OmpK showed protection against infection (Mao et al. 2007). Further, immunization of crucian carp and mice with *V. parahaemolyticus* VP1061 and VP2850 proteins induced a cross protective effect against *V. alginolyticus*, *A. hydrophila* and *Pseudomonas fluorescens* (Li et al. 2010a). OmpK, a homologous protein of the *Vibrio* species was administered to orange spotted grouper. Fish immunized by OmpK were able to survive infection from various strains of *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus* (Li et al. 2010b).

In sum, the above studies highlight the role of various gram-negative bacterial porins and few outer membrane proteins as vaccine candidates. Porins are able to stimulate T cell and B cell responses as well as offer protection against various gram-negative bacterial infections.

Conclusion

Outer membrane proteins are crucial for maintaining bacterial structure and homeostasis. These proteins are also important for gram-negative bacterial pathogenesis as they modulate host immune responses. Porins, a class of outer membrane proteins induce inflammatory responses in a range of host cells. They can also activate dendritic cells, T cells and B cells as well as shape adaptive immune responses. The signaling cascades activated by various porins have been delineated and their characterization has added to our knowledge on how they modulate host cell responses. Further, multiple porins have been reported for their vaccine potential and are undergoing further studies for their use as vaccines or adjuvants.

A number of patents have been filed since 2005 for the use of porins and outer membrane proteins of various gram-negative bacteria as vaccines or adjuvants. The use of *Salmonella* spp. OmpC and OmpF as adjuvant for influenza vaccine show improved immune response as compared to administration of influenza vaccine alone (Leclerc and Lopez 2010). Class 1 porins of *N. meningitidis* show significant immune stimulating capability and has the potential to be used as a vaccine for meningitidis (Seid et al. 2006; Paradiso et al. 2007; Van et al. 2007; Granoff et al. 2013). OmpK36 and its homologues from *K. pneumoniae*, *S.* Typhi, or *E. coli* open up a prospective in the diagnosis, treatment and prevention of *enterobacteriaceae* infection (Siu et al. 2013). The MOMP of *H. influenzae* and *C. trachomatis* show protective response against influenza/otitis media and Chlamydiophilia infections respectively (Berthet et al. 2011; Stephens and Kawa 2011). Besides porins, surface

protein of *Moraxella catarrhalis* and outer membrane vesicles of *V. cholerae* were successfully tested as vaccines (Chen et al. 2005; Camilli et al. 2014). All these studies highlight the necessity to examine porins and other outer membrane bacterial components for their adjuvant capacity and vaccine potential.

In conclusion, porins have emerged to have many more functions than previously believed and have the potential to be used for diagnosis and treatment of various gram-negative bacterial infections.

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