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Review Article

Host Receptor Immunomodulation in Response to *Shigella* Surface Antigens: An Insight for Vaccine Development

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Abstract: Shigellosis, caused by *Shigella* is the most common cause of bacillary dysentery. The disease is associated with high morbidity and mortality rate owing to its multiple drug resistance. Hence recovery from the disease would primarily depend on the development of an effective immune-modulator for strong mucosal immune response. The role of cellular immunity may be a critical factor in protection against shigellosis as *Shigella* remains an intracellular pathogen during most of its life-cycle. Development of a potent immunomodulator may provide strong and long-lasting immunity to shigellosis. In this review, we have attempted to highlight the disease dimension and its deviation due to the effect of various *Shigella* surface antigens that would help in the development of an effective immune response. Cellular innate immune modulation will be a new generation target for the development of mucosal candidate vaccines where proper receptor activation such as Toll-Like Receptors (TLRs), Cytokine Receptors (CyRs) and/or T-Cell Receptors (TCRs) on the host cell could be aimed at producing mucosal immunity. An effort has been made to better understand the effect of these immunomodulators against shigellosis by way of modulating the host immune mechanism to *Shigella* outer membrane component.

Keywords: *Shigella*, Surface Antigens, Host Cell Receptors, Immunomodulation, Immune Response

Introduction

Bacillary dysentery is a highly contagious enteric bacterial infection. It is the most important cause of high morbidity and mortality among infants and young children in both developed and developing countries worldwide. It has been estimated that about 200 million episodes occur per year in children up to 5 years of age, resulting in as many as 3-4 million deaths (Ferreccio *et al*., 1991; Lindberg and Pal, 1993). Among the entire toll of diarrheal diseases, five million people suffer annually from *Shigella* infection in developing countries and nearby about 700,000 individuals die from this infection (WHO, 2005; Kotloff *et al*., 1999). On an around 10% of entire child deaths below 5 years are responsible due to shigellosis (Ferreccio *et al*., 1991; WHO, 2005). Different *Shigella* species (*spp.*) and their serotypes vary in their geographical distribution- some being more prevalent than the other in a specific region in the world (Table 1). The disease is associated with poor hygienic standards and malnutrition. *Shigella* is usually transmitted directly from person-to-person by the fecal-oral route. *Shigella* can also be transmitted through food or water contaminated by human waste. 40% of adults and 20% of children develop infection from epidemic household contacts (CDC, 2001). The clinical signs of shigellosis range from mild diarrhea to severe dysentery with frequent passage of bloody, mucoid, small-volume stool accompanied by fever, abdominal pain and tenesmus at the acute stage (Keusch, 1986; Keusch *et al*., 1988). Clinical symptoms in elderly above 65yr are as severe as in children (Daniel and Lawrence, 2003).

Table 1. Geographical distribution of *Shigella* serotypes

Shigella species	*Serogroup*	Number of serotypes	Geographical distribution (predominant)
S. dysenteriae	A	15	Central America, South and South-East Asia, Sub-Saharan Africa
S. flexneri	B	14	Asia, Europe, Latin America
S. boydii	C	20	South Asia, Sub-Saharan Africa, Middle East
S. sonnei	D	1	East Asia and Pacific, Australia, East Europe, Middle East, North and Latin America

Sometimes, it creates havoc in elderly than in children for the development of *Shigella* bacteremia (Morduchowicz *et al*., 1987). *Shigella* is extremely infectious bacteria and ingestion of just 10 organisms is enough to cause severe diarrhea and dehydration (Dupont, 1989). Usually, the incubation period varies from 6 to 96 hours with the infecting strains. The infection is self-limiting in healthy hosts but in malnourished children, the disease persists over days to weeks or even last for several weeks and months.

Infection caused by *S. dysenteriae* 1 strain producing Shiga toxins, a potent cytotoxin have a tendency towards a serious severity like hemolytic uremic syndrome, which has a high mortality rate (Dupont, 1990) as compared to the other non-toxin producing strains.

Many cases of bacterial dysentery improve without antibiotic treatment. In fact, in healthy individuals, the infection may subside on its own within 4-7 days, with home remedies and oral rehydration. Therefore, drugs are needed only for the treatment of moderate or severe disease. Choice of antibiotic is based on the type of bacteria found in the geographical area and on laboratory test results. WHO (1997) recommends ampicillin, sulfa derivatives such as Trimethoprim-Sulfamethoxazole (TMP-SMX) trade name Bactrim, or fluoroquinolones such as Ciprofloxacin, which are currently used in adults; however these drugs are not approved by FDA for use in children).

Antibiotic is virtually advised for every treatment during shigellosis (Oldfield and Wallace, 2001; Dhodapkar *et al*., 2008). First line choice of antibiotic for *Shigella* therapy is the quinolone family of drugs. Murphy *et al*. (1993) suggested that ciprofloxacin should be used orally in adult shigellosis. Another study showed that loperamide, an anti-motility agent, leads to quicker improvement without prolonging *Shigella* elimination. Azithromycin has similar efficacy to ciprofloxacin (82% for azithromycin and 89% for ciprofloxacin) (Khan *et al*., 1997; Taneja, 2007) and showed intracellular penetration and moderate *in vitro* *Shigella* activity. With increasing quinolone resistance, other agents have been explored, such as first- and second-generation cephalosporins that are active *in vitro* but have proven to be a disappointment in clinical use (Oldfield and Wallace, 2001). According to Anh *et al*. (2001) about 80% of the *Shigella* strains tested were resistant to ampicillin, chloramphenicol, oxytetracycline, trimethoprim and sulfonamides. Changing patterns of antimicrobial susceptibility of such *Shigella* serotypes isolated from children with acute diarrhea suggest that multidrug resistance causes over a million deaths in developing countries (Mamatha *et al*., 2007). Chloramphenicol or nalidixic acid has now been recommended for severe dysentery, as it is low-cost, effective and safe for children. However, some strains of *Shigella* showed 3-5% resistance to nalidixic acid and norfloxacin (Anh *et al*., 2001). In Fiji Islands, only intravenous antibiotics sensitive to *Shigella* like, ceftriaxone and cephalothin could be advised in case, if the patients are unable to take oral drugs (Watson, 2001) whereas, cephalothin was found to have 57.1% resistance to all *Shigella* sero-groups in Ethiopia (Mache, 2001). Gentamycin, polymyxin B and nalidixic acid were found to be the drugs of choice for shigellosis. Continuous surveillance of multidrug resistant strains is very important in order to understand the changing patterns of antibiotic susceptibility (Doyle, 2014; CDCR, 2015; Srinivasa *et al*., 2009) as well as mutations in serogroups. Change in drug resistance pattern is associated with the change in serogroup genetic makeup. Thus, antibiotic treatments following development of resistance sometimes may develop inability to produce antibodies in patient and induce immunity.

Over the past few decades, the numbers of multi-drug resistant *Shigella* strains have progressively increased treatment failure and mortality (Doyle *et al*., 2013; WHO 2014). Thus, an alternative approach using immunomodulators could be a useful strategy to treat patients with multi-resistant *Shigella* infection. The use of these immunomodulators could possibly trigger a biased antigen-specific signal to increase immune functions.

Specific and type-specific immunity to *Shigella* infection is acquired after dysentery but is relatively weak and for a short duration. Therefore, the disease may recur many times due to drug resistance and in some cases, may become chronic (Hens *et al*., 2005). Targeting host cellular receptors in order to induce type-specific humoral or cellular immune response could be beneficial in achieving immunity to shigellosis and overcome the disease burden in developing countries. Immune responses against *Shigella* surface antigens like Lipopolysaccharide (LPS) molecules, Outer Membrane Proteins (OMPs) and the invasion plasmid-coded proteins (Ipa's) are specific and most pronounced (Oaks *et al*., 1986; Oberhelman *et al*., 1991). Changes in the secretory immune system of the intestinal mucosa have been shown to cause primary mucosal immunity. These secretory antibodies could help in the activation of intra-epithelial lymphocytes (Phalipon *et al*., 2002), which could possibly trigger

release of pro-inflammatory cytokines such as Interleukin (IL)-1β, IL-6, Transforming Growth Factor (TGF)-β and Interferon (IFN)-γ that are associated with the degree of infection (Islam *et al*., 1997; Raqib *et al*., 1995a). However, the levels of receptor expression are down-regulated and inversely correlated with the severity of the disease (Raqib *et al*., 1995b). It is now being said that many inflammatory reactions occur in order to acquire specific immunity against shigellosis and are mediated by a broad range of cytokines released from the cytokine-producing cells.

Local antibodies against LPS and Ipa have been detected in intestinal secretions of *Shigella*- infected monkeys (Dinari *et al*., 1987; Oaks *et al*., 1986) and patients (Oberhelman *et al*., 1991). Most of the epidemiological and clinical data suggest that increased levels of serum antibodies are good indicators of natural or experimental stimulation by immuno-dominant antigens and in turn could induce effective immunity (Cohen *et al*., 1992; Oberhelman *et al*., 1991). An approach towards the immunoprophylactic measures against shigellosis may be achieved by way of elucidating a relation between pathogenesis (Navarre and Zychlinsky, 2000; Perdomo *et al*., 1994; Chakraborti and Sinha, 1994) and host immune mechanism using bacterial outer membrane components such as LPS, Ipa's and OMPs as potent immunomodulators or by modulating cellular innate system targeting specific receptors such as Toll-Like Receptors (TLRs), Cytokine Receptors (CyRs) and/or T-Cell Receptors (TCRs).

Shigella Surface Antigens: Immunomodulators

Variations in the *Shigella* surface antigens make it most challenging for the development of candidate vaccine (Ashkenazi *et al*., 1999; Cohen *et al*., 1988; Robbins *et al*., 1992). A strategy to develop pentavalent anti-O-antigens from five different *Shigella spp.* was undertaken to provide global immunity to *Shigella* infection (Iankov *et al*., 2004). Although there has been increased interest of understanding the protective efficacy of a single- or multi-serotype OMPs (Mitra *et al*., 2012), their sensitivity and specificity to species-specific infection needs to be considered. Several experimental evidences have shown the generation of humoral as well as cellular immune response against various *Shigella* surface antigens and/or super antigens (Acha-Orbea, 1993; Beckman *et al*., 1994; Herman *et al*., 1991; Witkowska *et al*., 1986). A strong antibody response to LPS and Ipa antigens has been reported earlier (Cam *et al*., 1992; Oberhelman *et al*., 1991; Passwell *et al*., 1995) that confers protection against shigellosis (Cohen *et al*., 1988; Mitra *et al*., 2012; Passwell *et al*., 1995). Two novel virulence loci, *mxi A* and *mxi B*, in *S. flexneri* 2a facilitating excretion of IpaB have been shown to modulate protection against the disease (Andrews *et al*., 1991).

Disease dimension and its deviation due to the effect of these antigens have not been systematically analyzed in order to have an effective humoral as well as cellular immunity to shigellosis. LPS-induced T-cell responses to produce cytokine as well as other cytokine-producing cell responses were also described earlier (Sieling *et al*., 1995). In contrast, these LPS molecules as an antigen itself cannot induce an effective cellular immune response since LPSs are T-independent antigens which require CD1 molecules to be recognized by T-cells (Beckman *et al*., 1994; Sieling *et al*., 1995). Besides, LPS and OMPs also play an important role in adherence, invasion, serum resistance and resistance to phagocytosis (Buchanan and Pearce, 1979). Moreover, OmpA of *S. flexneri 2a* has been found to induce a significant protective immune response in a mouse model (Pore and Chakrabarti, 2013; Pore *et al*., 2011).

Many strategies have been implemented to mitigate the pathogenicity of *Shigella* by gene manipulation in lipid A biosynthesis e.g., *htrB* and *msbB* (D'Hauteville *et al*., 2002). Serological tests have also revealed high cross-reactivity among these surface components of different groups of gram-negative bacteria and their species (D'Hauteville *et al*., 2002; Peterfi *et al*., 2007). Despite their strong immunogenicity, there are many limitations in using these candidates as a vaccine (Kaminski and Oaks, 2009; Kuehn and Kesty, 2005). One of the major drawbacks is that their specificity is limited to a particular species or sub-species rather than inducing global immune response to the disease. Indeed there is a need to identify a strong immuno-dominant component to trigger the host defense system against shigellosis. Most of the identified proteins have a univalent epitope and essentially participate in T-cell activation for antibody synthesis via peptide-H2 complex. Thus, proteins may be considered as T-cell dependent immunogens that can possibly generate exclusive antigen-specific immunoglobulins (Pore *et al*., 2009). Moreover, *ShiA*, the product of a novel Open Reading Frame (ORF), has also been shown to reduce inflammatory responses where T-cells play a major role in achieving immunity to shigellosis (Ingersoll and Zychlinsky, 2006). A genetic mutant of ShiA (Δ*shiA*) has been shown to induce polymorphonuclear cell migration resulting in increased inflammatory response (Ingersoll and Zychlinsky, 2006; Peterfi *et al*., 2007). It is now suggested that *ShiA* could be used to modulate immune system (Ingersoll and Zychlinsky, 2006). Such purified antigenic protein fraction or factors in the ORFs may facilitate immunomodulatory effects in monitoring antigenic-specific signals and ascertain its immunoprophylactic nature against such infection.

Immunomodulatory Effect of Surface Antigens

Earlier many *Shigella spp.* and their surface components have been identified and used as mucosal vaccine candidates (Barman *et al*., 2011; Iankov *et al*.,

2004; Kaminski and Oaks, 2009; Kuehn and Kesty, 2005; Mitra *et al*., 2012; Pore *et al*., 2009) but their immune-stimulatory effects were less defined. Lack of immuno-stimulatory features of the target candidates often does not elicit strong mucosal immunity (Borisova, 1999). Immunogenicity of the candidate vaccines is less described since available surface antigens exhibit weak epitope determinant sites and fail to induce antigen-specific responses. Immunomodulatory molecules are often predominantly derived from the pathogens that efficiently activate immune system leading to acquired host adaptiveness. There are yet many unknown facts that need to be explored before rationalized development of candidate vaccine for safety, immunogenicity and its efficacy. Thus it may require two basic components to verify the vaccine efficacy: First, an appropriate animal model to test the safety and immunogenicity of the candidate (Katz *et al*., 2004; Robbins and Schneerson, 1990) and, secondly, the presence of an appropriate carrier molecule (Beckman *et al*., 1994; Sieling *et al*., 1995).

Despite many efforts, there is a profound lack of immunomodulatory effect of the available surface antigens. The immunomodulatory effect of *Shigella* porin has been described to mediate antibody dependent B-cell responses (Alurkar and Kamat, 1997; Roy and Biswas, 1996). Small dose of *S. flexneri* OMPs has also been found to induce humoral as well as cell-mediated immune responses (Czarny *et al*., 1992; Sinha *et al*., 1992), whereas a higher dose suppresses Delayed Type Hypersensitivity (DTH) in mice (Chakrabarti and Sinha, 1994). Immuno-dominant components such as 38, 34, 23 and 20kDa of *S. flexneri* OMPs were also found to induce protective immune responses against shigellosis (Mukhopadhaya *et al*., 2003). Nevertheless, 34 kDa OMPs of *S. flexneri* 2a was found to increase IL-12p70 activity by macrophage (Ingersoll and Zychlinsky, 2006) together with increased levels of serum IgG and IgA and other cytokines in both systemic and mucosal conditions (Arranz *et al*., 1992; Pore *et al*., 2011). Many subunits of conjugated vaccines have also been shown to cause limited peripheral antibody production (Camacho *et al*., 2011; Hartman *et al*., 1994; O'Hagan and Valiante, 2003; Robbins *et al*., 1992).

Humoral Immune Response to *Shigella* Surface Immunomodulators

Immunity to *Shigella* or other enteric pathogens is primarily mediated by secretory IgA antibodies (Islam *et al*., 1997; Phalipon *et al*., 2002; Pore *et al*., 2011). Sequential changes of humoral immune response within the antibody class are most important to determine immunogenicity of the antigens (Islam *et al*., 1995a; 1995b). Studies on humoral immunity in natural *Shigella* infections (Dinari *et al*., 1987; Witkowska *et al*., 1986) revealed that LPS, OMPs and IpBs play an important role in pathogenesis (Ashkenazi *et al*., 1999; Cohen *et al*., 1991; Perdomo *et al*., 1994) and subsequent protection (Beckman *et al*., 1994; Czarny *et al*., 1992; Robbins and Schneerson, 1990). Immunomodulatory effect of OMPs on humoral immune response was shown by the number of Plaque-Forming Cells (PFCs) producing anti-Sheep Red Blood Cell (SRBC) antibodies (Czarny *et al*., 1992). Besides such envelope antigens, a number of Ip-related antigens represent major immune-stimulating moieties as dysenteric patients react with a strong and consistent Ipa-specific antibody response (Andrews *et al*., 1991; Oberhelman *et al*., 1991). Delayed and reduced adaptive humoral immune response was seen in children with shigellosis compared to adults (Raqib *et al*., 2002). However, earlier studies showed an antigenic relationship between major OMP and IpaB (Peterfi *et al*., 2007; Witkowska *et al*., 1986). A strong IgG-specific cross-reactivity of human convalescent sera was also found between these antigens (Iankov *et al*., 2004; Martinez-Becerra *et al*., 2012; Sieling *et al*., 1995). The kinetics of serum IgG responses against *Shigella* IpaB and IpaD have been studied after each course of immunization and was shown to have differential changes in mucosal IgA or IgG Antibody Secretory Cells (ASCs) suggesting a protective role of such secretory proteins (Andrews *et al*., 1991; Arranz *et al*., 1992; Camacho *et al*., 2011).

Importantly, the development of an effective candidate vaccine against infection requires critical analysis of the local and systemic immune response (Raqib *et al*., 2002). Heat killed or live attenuated *S. flexneri* 2a strain SC602 was used in a community based vaccine trial and 50% of the volunteers were found to have an increased IgA response to *S. flexneri* LPS (Katz *et al*., 2004; Mukhopadhaya *et al*., 2003; Robbins *et al*., 1992). The magnitude of the mucosal immune response to LPS was successfully elicited only in 19% of the volunteers (Martinez-Becerra *et al*., 2012; Turbyfill *et al*., 2000). Anti-LPS and anti-Ipa antibodies as well as antibody-secreting cells were also evaluated in volunteers orally vaccinated with non-Shiga toxin producing *S. dysenteriae* (Hartman *et al*., 1994; Robbins *et al*., 1992; Samandari *et al*., 2000). Further, whole cell lysate-envelope fraction and OMPs were detected by the antisera against heat-killed *S. flexneri* 2a (Hartman *et al*., 1994). Correlation between anti-LPS antibody titers and the level of protection to O-polysaccharide haptens of *S. dysenteriae* 1, *S. flexneri* 2a, or *S. sonnei* conjugated to various protein carriers have also been shown to possess parenteral, single-shot and low toxicity candidate vaccine (Harandi *et al*., 2003; Robbins *et al*., 1992; Robbins and Schneerson, 1990). These conjugated proteins induced mucosal humoral immunogenicity of the O-antigen against the homologous challenge (Ashkenazi *et al*., 1999). Such antibodies have also shown to be protected in invasion of *Shigella* into Coca-2 cells and may be curative (Chowers *et al*., 2007; Robbins *et al*., 1992). The high antibody IgG concentration induced by these conjugate vaccines in volunteers who did not develop shigellosis

suggests that there is an association between serum antibody titer and protection.

Cell-Mediated Immune Response to *Shigella* Surface Immunomodulators

Current knowledge on host immune mechanism is limited to mucosal antibody response. Role of cell-mediated immune responses in host defense system is yet to be understood. Therefore, factors involved in pathogenesis and patterns of the immunological responses during infection and its recovery must be considered in the development of a potent immunomodulator. Identification of immunogenic molecules either derived from a *Shigella* pathogen to modulate host T-cell functions (Beckman *et al*., 1994; Phalipon and Sansonetti, 2007) or detect some potential cellular and molecular targets against these antigenic molecules in order to acquire protection (Sieling *et al*., 1995; Sinha *et al*., 1992). Immunomodulatory effect of Shiga toxin released by *Shigella* have been shown to stimulate mononuclear cells including $CD4^+$ or $CD8^+$ T-cells to proliferate (Samandari *et al*., 2000) and produce cytokine (Tesh *et al*., 1994). An increasing number of CD4 cells than that of CD8 cells in the lamina propria of *Shigella* infected patients with significant expression of Human Leukocyte Antigen (HLA)-DR revealed its significance in the induction of cell-mediated immune responses during acute shigellosis (Islam *et al*., 1995a; Raqib *et al*., 1994). Such cellular activation through Antigen Presenting Cells (APCs) may provide host defense against shigellosis elicited by vaccination (Bagchi and Sinha, 2004; Cohen *et al*., 1992). It is very well known that IpaB mediates apoptosis in macrophages induced by *Shigella* (Hilbi *et al*., 1998; Navarre and Zychlinsky, 2000; Perdomo *et al*., 1994) through IL-1β-regulated caspase-1 activation (Hilbi *et al*., 1997). Thus Ipa's could be targeted as an immunomodulator since there was a robust mucosal antibody production as well as T cell-mediated immunity (Beckman *et al*., 1994; Cam *et al*., 1992; 1993; Oberhelman *et al*., 1991). Particularly, IpaB has been shown to have a protective role against lethal pulmonary infection by *S. flexneri* or *S. sonnei*. Thus these secretory Ipa antigens, IpaB and IpaD, have been suggested as potent immunomodulators which may provide cross-protection (Peterfi *et al*., 2007; Turbyfill *et al*., 2000). Synergistic effect of IpaB and IpaD has been shown to activate APCs and differentiate T-cells into Th1 (O'Hagan and Valiante, 2003; Robbins *et al*., 1992). Nevertheless, due to high cross-reactivity of these OMPs to Ipa's of the different groups of Gram-negative bacteria (Peterfi *et al*., 2007; Rabbi *et al*., 2008), these OMPs of *Shigella* and other genera of enterobacteriaceae have been shown to induce protection not only against homologous but also heterologous infection in a mouse model (Czarny *et al*., 1990). Although the OMPs are highly immunogenic and protective against lethal infection of *Shigella* (Adamus *et al*., 1980; Mulczyk *et al*., 1981), this results in immunosuppression of humoral as well as cellular responses (Mulczyk *et al*., 1987; Witkowska *et al*., 1986) in murine models. Hence, there is a need to develop cellular immunity of the host, which may be a critical factor in protection against shigellosis.

Immunity to *Shigella* infection is mainly characterized by sensitized T-cells (Islam *et al*., 1995a; 1996; Raqib *et al*., 1994) since there is increased inhibition of DTH (Mukhopadhaya *et al*., 2003; Mulczyk *et al*., 1987) and inflammatory cytokines (Raqib *et al*., 1995a; 1995b; 1995c; Way *et al*., 1998). Since protective immunity to shigellosis is delayed, patients may thus develop severe clinical complications (Azim *et al*., 1996; Raqib *et al*., 2002). Subcellular basis of *Shigella* destruction as well as immunological basis of T-cell suppression either in acute or chronic *Shigella* infection is not yet completely understood. However, studies on T-cell subsets suggest a biased induction of Th2 (Azim *et al*., 1996; Islam *et al*., 1995b). Therefore, lack of protective Th1 response during most of the experimental studies suggests a nature of T-cell suppression (Barman *et al*., 2011). Present scenario demands an alternative approach to our existing concept on cell-mediated immunity in shigellosis. Cellular immunity at the mucosal surface has been shown in the regulation of effector immune cell functions where $CD8^+$ T-cell response to live attenuated vaccines are thought to contribute significantly to cross-protection against various influenza strains (Sun *et al*., 2011). On the contrary, effector cell functions of CD8+ T-cells have also failed to induce protection against shigellosis (Jehl *et al*., 2011). $CD4^+$T cells have also been shown in *Shigella* induced by outer membrane proteins (Bagchi and Sinha, 2004; Pore and Chakrabarti, 2013) through activation of either classical Toll-Like Receptor 2 (TLR2) or NLR innate signaling (Biswas *et al*., 2009; 2008; Phalipon and Sansonetti, 2007). Since TLRs activate cellular innate immunity, modulation in TLR2 signaling by porins may enhance $CD4^+$ helper cell activity in order to acquire antibody response via APC and B-cell interaction (Biswas *et al*., 2009; 2008). However, activation of these helper cells together with CD25 enhances Interleukin-2 (IL-2) and Macrophage Inhibitory Factors (MIF) release instead of Activating Factors (MAF) required for T-cell regulation during acute stage of *Shigella* infection (Bagchi and Sinha, 2004). The presence of HLA-DR antigen with $CD4^+$ helper T-cells in the gut mucosa of acute patients (Raqib *et al*., 1994) as well as T-cell restricted MHC-class II responses to major antigenic *Shigella* OMPs in immunized mice (Bagchi and Sinha, 2004; Pore and Chakrabarti, 2013) suggests a significant role of these helper T-cells in protection through activation of APCs. Furthermore, increased proliferation of $CD4^+$ T-cells together with IL-2 in such immunized mice following infection was due to selective $CD8^+$ T-cell apoptosis by caspases-1 and IL-18 (Bagchi *et al*., 2010a). Overall, study suggested that recruitment of specific memory

Th1-cells and their activation during *Shigella* infection following immunization may play a crucial role in the mitigation of the disease.

Patterns Recognition Receptors (PRRs)-Specific Immunomodulation During *Shigella* Infection

PRRs are not only specific to pathogen associated molecular patterns but also linked with Damage-Associated Molecular Patterns (DAMPs) induced by ceramides (synthesized due to bacterial toxins or surface components). These PRRs are critical regulators of innate immune system and are present on almost every cell type including APCs and M-cells. Bacterial outer membrane components such as Bacterial Lipoproteins (BLPs) or LPS are recognized by these PRRs such as TLR4 (Takeuchi *et al*., 2000). BLPs are characterized by a unique N-terminal lipo-amino acid also known to engage TLRs (Takeuchi *et al*., 2000). Since BLP is produced by all bacteria, it has important implications in immune system specific to pathogens thus making it a novel immunomodulator for therapeutic consideration. Immunomodulation of TLRs due to these bacterial outer membrane or secretory components have been studied in different *in vitro* conditions (Alurkar and Kamat, 1997; Wetzler *et al*., 1996) and has been shown to be involved in bacterial translocation leading to the activation of classical inflammatory responses through Nod-Like Receptors (NLR) innate signaling (Aliprantis *et al*., 2001; Phalipon and Sansonetti, 2007). Development of an effective immunomodulator using LPS as an adjuvant might trigger innate signaling via TLRs (Aliprantis *et al*., 2001; Alurkar and Kamat, 1997; Gribar *et al*., 2008). Recently, it has been reported that TLR activation is dependent on the number of acylated groups present in lipid A (Gribar *et al*., 2008; Rallabhandi *et al*., 2008). *S. flexneri* 2a elicited comparatively weaker TLR4-mediated innate immune response than *E. coli* LPS due to penta-acylated group of lipid A (Rallabhandi *et al*., 2008). Hence it is suggested that *Shigella* LPS-induced apoptosis is dependent on TLR2 (Aliprantis *et al*., 2001; 2000; Alurkar and Kamat, 1997; Rallabhandi *et al*., 2008), whereas it is independent on TLR4 (Aliprantis *et al*., 2001; Kaminski and Oaks, 2009; Ray and Biswas, 2005; Suzuki *et al*., 2005) through activation of IL-1β and IL-8 (Aliprantis *et al*., 2000; Toussi *et al*., 2012). On the other hand, porins of *Shigella* have been shown to activate TLR2 or TLR6 and modulate B-cell functions to maintain mucosal humoral response by $CD4^+$ T cells (Ray *et al*., 2004). Such TLR adjuvant had induced B2-cells to produce IgM and IgG 2a (Ray and Biswas, 2005; Ray *et al*., 2003) and develop $CD4^+$T-cell memory response (Biswas *et al*., 2009). Activation of TLR2 and TLR6 innate signaling promotes adaptive signaling via activation of CD80-CD86 (Ray *et al*., 2003; 2004) following effector T-cell functions (Aliprantis *et al*., 2001; Ray *et al*., 2004). These studies suggest that modulation of TLR or NLR innate signaling in adaptive response may last longer in the maintenance of cellular homeostasis in order to achieve protection against shigellosis (Phalipon and Sansonetti, 2007; Pore and Chakrabarti, 2013). These novel findings further provided drive for developing new candidate vaccines against mucosal pathogens that target TLRs.

CyRs-Mediated Immunomodulation During Shigellosis

Protective immune cell responses to the infection are provided through antigen specific signals in which cytokines and co-stimulatory molecules play an important role in the development of immunity (Chakrabarti and Sinha, 1997; Chowers *et al*., 2007; Le-Barillec *et al*., 2005; Raqib *et al*., 1994). During shigellosis, many inflammatory reactions are triggered by a wide range of cytokines mediated by innate immune system (Cohen *et al*., 1992; Raqib *et al*., 1995a). Inflammatory cytokines such as IL-1, IL-3, IL-4, IFN-γ, TGF-β and TNF-α etc. have been identified during acute *Shigella* infection (Bagchi *et al*., 2010b; Raqib *et al*., 1995a) and have been shown to increase at the convalescent stage (Raqib *et al*., 1995b; 1995c). *In vivo* and *in vitro* studies have shown IFN-γ induced innate resistance to primary *Shigella* infection (Cohen *et al*., 1991; Way *et al*., 1998). However, the path for a selective response is undefined which is possible only when the responses are being acquired through related antigens. Cytokines and their receptors may possibly gain an access in search of a selective pathway by generating adaptive immune responses. While their role in host defense mechanism are not well defined, it is known that the primary cytokines like MIF and MAF are released from the macrophages and are essential for the activation of class-II antigens with T-cell (Bagchi and Sinha, 2004). During T-cell activation, pro- and anti-inflammatory cytokines such as IL-2, IL-4, IFN-γ and IL-10 are released (Azim *et al*., 1996; Bagchi and Sinha, 2004; Islam *et al*., 1995a; Raqib *et al*., 1994) - this might help in CyRs activation resulting in memory T-cell activation and differentiation. Earlier, the concentration of IL-2 induced by a major OMP was determined in natural infection (Chakrabarti and Sinha, 1997). Elevation in IL-2 and IFN-γ levels in mice immunized with major OMPs suggested that T-cell activation was induced to recall its memory against the antigen (Bagchi and Sinha, 2004; Herman *et al*., 1991; Toussi *et al*., 2012). It has been shown that IFN-γ production not only by macrophages but also by T-cells may be considered as a surrogate biomarker for mucosal disease (Samandari *et al*., 2000; Witkowska *et al*., 1986) suggesting a role in cellular immunity against *Shigella* infection. Synthesis of IFN-γ by T-cells appeared to increase when IpaB was administered alone (Cohen *et al*., 1991; Witkowska *et al*., 1986) or together with IpaD, reflecting a robust cell-mediated immunity (Le-Barillec *et al*., 2005;

Samandari *et al*., 2000). However, deficiency of IFN-γ may aggravate the disease progression as there has been an increase in the number of intracellular *Shigella* further suggesting that IFN-γ helps in controlling the infection (Way *et al*., 1998). Thus activation of IFN-γ production by targeting its receptors may suppress the effect of primary inflammatory response due to generation of MIF by stimulated macrophages (Bagchi and Sinha, 2004). Thus, modulation in CyRs on the cell surface may be a better approach to alleviate inflammatory responses to antigens. In the presence of anti-IFN-γ antibody, killed *Shigella* bacteria induced elevated IL-2 and IFN-γ at the early stage of infection, but at the later stage, these IFN-γ levels were diminished (Sinha and Bagchi, 2005). Data suggests that increased level of IFN-γ production during acute phase may facilitate the up-regulation of antigen processing by means of generating high levels of oxygen free radicals. At the later stage, it was assumed that processed antigens are presented by APCs to T-cell through MHC-class II molecules and release protective cytokines (Bagchi and Sinha, 2004; Sinha and Bagchi, 2005; Witkowska *et al*., 1986). Therefore, immunomodulation of such targeted CyRs may represent one facet of cellular immunitythat could be important in resistance to *Shigella* organisms.

TCR Ligand-Specific Immunomodulation Against *Shigella* Infection

Immunosuppression in shigellosis is mediated by intra-epithelial memory T-cells. Immunosuppressive mode of *Shigella* infection is due to poor early activation or lack of these antigen-reactive T-cells. Regulatory activities of these memory T-cells at the mucosal site is still not clear. There have been several reports on polyclonal T-cell activators like anti-CD1, anti-CD2, anti-CD3 and anti-IL-2 which are often used either to induce lymphoid cell proliferation or cytokine secretion (Moreno-Lafont *et al*., 2003; Nguyen *et al*., 1995; Sieling *et al*., 1995; Sinha and Bagchi, 2004). Anti-CD3 (TCR ligand) identified on T-cell subsets has been not only known to activate and induce memory T-cell but also shown to promote the synthesis of new cytokines (Sinha and Bagchi, 2004). It is also known that activated memory T-cells play an important role in mediating antigen specific response to bacteria. In addition, memory T-cells have been shown to determine IFN-γ levels which strongly reflects their role in regulation of Th1 cytokines (Bagchi *et al*., 2010a; Sinha and Bagchi, 2004). Activation of TCRs by TCR ligand or OX40 ligand (a co-stimulator of Th1-cell) contributes to maintain effector T-cell function as well as to develop memory against antigen-specific stimulation (Bagchi *et al*., 2010a; Maxwell *et al*., 2000) via activation of anti-apoptotic Bcl-xl expression of Bcl-2 family proteins (Rogers *et al*., 2001). The TCR ligand, anti-CD3 antibody, was also found to trigger effective T-cell function against *Shigella* antigens and elicit adaptive immune response against shigellosis (Bagchi *et al*., 2010a). This suggests that TCRs might ameliorate the property of *Shigella* surface antigens as a potential immuno-dominant candidate.

Shigella alone may not explain their immune stimulating moieties due to poor activation of reactive T-cells. During the course of infection, therefore, regulatory activities of the memory T-cells are needed to be considered to activate biased signals for antigen-specific receptors. During maturation and activation of TCR, IL-2R expressed on T-cell is required for IL-2 growth factors via co-receptor molecule such as CD28 leading to IL-2 and IFN-γ secretion towards the cellular target. In the presence of co-stimulatory signals, TCR ligands may also activate memory T-cells via TCR activation (Bagchi and Sinha, 2005; Sinha and Bagchi, 2004). Conversely, in the absence of these signals, TCR cells develop tolerance (Barrett *et al*., 1993). TCR activation by anti-CD3 ligand stimulation in murine T-cells has been shown to induce IL-2 secretory T-cells and requires co-stimulatory signals for further activation and proliferation of memory T-regulatory cells (Moreno-Lafont *et al*., 2003; Sinha and Bagchi, 2004). T-cell activation up-regulates protein kinase-C-mediated phosphorylation in $CD4^+$ T-cells which enhances IL-2 production, lipid peroxidation as well as formation of reactive oxygen species (ROS) (Bagchi and Sinha, 2005; Sinha and Bagchi, 2004). Increased production of IL-2 enhances its receptor (CD25) activation on macrophages (Sinha and Bagchi, 2004). The ligand-induced TCR activation might suppress the naïve or memory $CD8^+$ T-cells resulting in $CD4^+$ T-cell selection (Bagchi *et al*., 2010b). On the other hand, TCR ligand- induced T helper cell activity was shown to produce IgG and or IgM by B-cells (Bagchi *et al*., 2010b). Data suggests that antibody can provide B- cell mediated adaptive immunity. Adaptive immunity to intracellular pathogens is mediated by sensitized T-cells in which both $CD4^+$ and $CD8^+$ T cells contribute to the protection against subsequent challenges (Bagchi *et al*., 2010a; Phalipon and Sansonetti, 2007) via Phosphoinositide 3-Kinase (PI3K) pathway (Bagchi and Sinha, 2005). Thus, anti-CD3 antibody would be an immuno-stimulatory molecule to induce adaptive immune responses through TCR activation. Such ligand-induced T-cell modulation may efficiently promote the ability of the used (specific) antigen in achieving immunity to shigellosis.

Conclusion

The antibody response as an approach to evaluate the immune mechanism is responsible for immunity induced in vaccinated animals. The antigenicity to immunomodulators either from *Shigella* surface antigens or other adjuvants delivered as a carrier molecule would primarily depend on the strength and amount of cell-specific signal generated against *Shigella* infection.

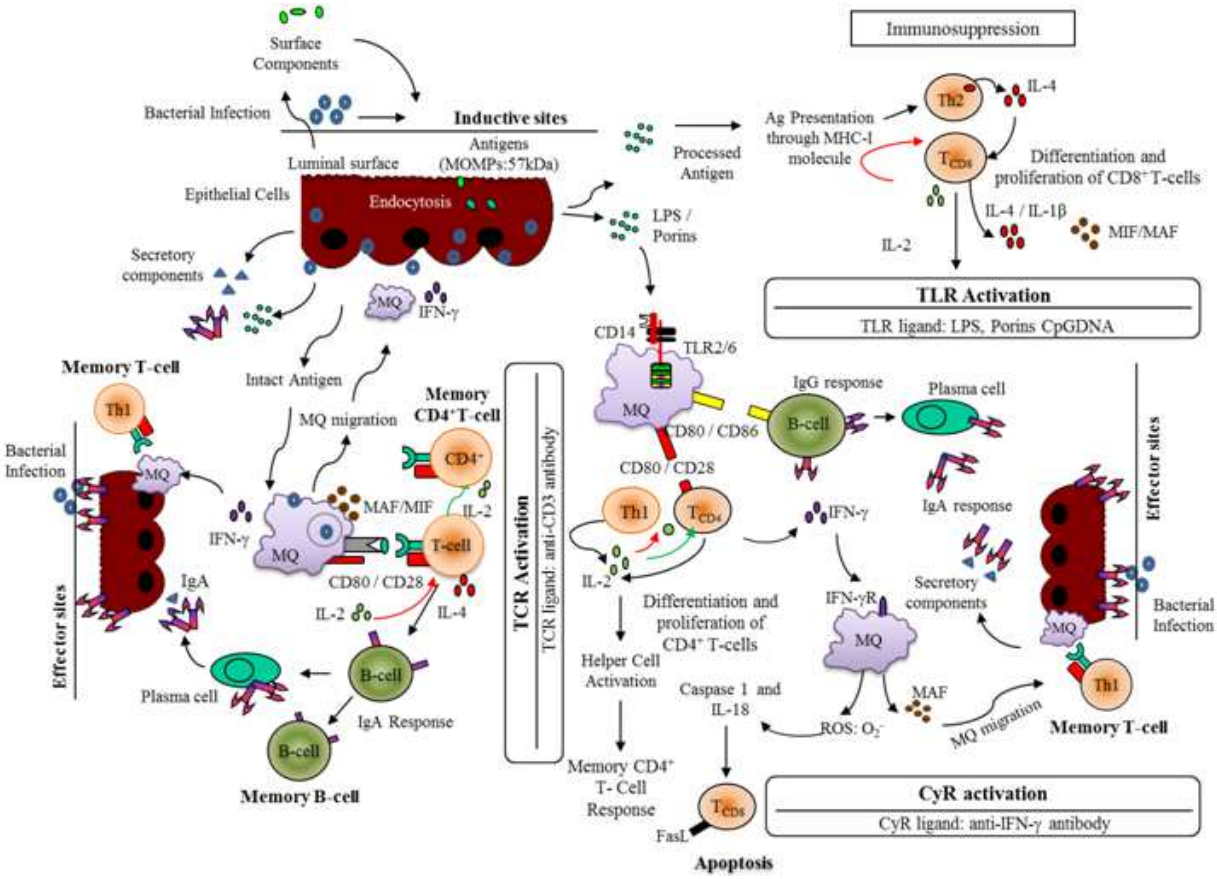

Fig. 1. Schematic diagram represents the cellular basis of receptor immune-modulation due to *Shigella* surface immunomodulators at the mucosal lining via activation of Pattern Recognition Receptors (PRRs) such as Toll-Like Receptors (TLRs), T-Cell Receptors (TCRs) and Cytokine Receptors (CyRs). Antigen specific cellular immune response at the mucosal sites, where *Shigella* Major Outer Membrane Proteins (MOMPs) such as 57 kDa antigen are exposed to the inductive sites to either trigger Ig secretory components, or help in phagocytosis by Macrophage (MQ). Antigens are now processed and presented to T-cell through Major Histocompatibility (MHC) class I or II molecules presents on MQ. During this process, Interferon (IFN)-γ facilitates the up-regulation of antigen processing by means of generating high level of oxygen free radicals such as superoxide anions (O_2^-) and MQ Activation Factors (MAF) instead of MQ Inhibition Factors (MIF) (Bagchi and Sinha, 2004). These primary cytokine, MAF helps MQs to migrate at the infection sites by inhibiting MIF released at very initial hours. During this process, T-cells releases Interleukin (IL)-2 or IL-4, helps in cell proliferation, differentiation and growth in autocrine (red line) or paracrine (green line) fashion. The fate of the T cell population, $CD4^+$ or $CD8^+$ T-cell selection is depending on the strength of primary signal lineage therefore generated helper or suppressor cell activity promoted by either IL-2 or IL-4 respectively. *Panel A*: IL-2 promoted the helper T-cell activity through MHC-II molecule while IL-4 promoted suppressor T-cell ($CD8^+$) through MHC-I molecule and cause immunosuppression. *Panel B:* Adjuvants, such as porins activates innate signaling via expression of TLR2 and TLR6 on the MQs and interact with $CD4^+$ specific T-cell (Biswas *et al*., 2009; Biswas *et al*., 2008), which later interact with activated B-cells through secondary signal (CD80/CD86) (Ray *et al*., 2003) as a result of which confers mucosal immunity by production of immunoglobulin (Ig) G and IgA (Toussi *et al*., 2012) specific for antigen at the effector site. *Panel C:* Cytokines such as IFN-γ may also play an important role in shigellosis as there was an overproduction of these IFN-γ during the course of the disease which might not be beneficial to substantiate the progression of the disease (Raqib *et al*., 1995; Raqib *et al*., 1995). Thus modulation in CyR by using anti-IFN-γ antibody to internalize its effect might be helpful to protect from its overproduction (Moreno-Lafont *et al*., 2003). *Panel D:* Immunomodulation of TCR upon its ligand (anti-CD3 antibody) stimulation activates secondary signals for CD80/CD28 (Bagchi and Sinha, 2005; Bagchi *et al*., 2010), but in absence of these signals, TCR cells develop tolerance (Barrett *et al*., 1993). TCR ligand further stimulates and promotes proliferation of IL-2 specific memory T-cells (Bagchi *et al*., 2010; Nguyen *et al*., 1995). T-cell ligands are activated when $CD4^+$ T either interacts with their receptors on B-cell to generate antigen specific signals for the production IgG2a/2b antibody (Maxwell *et al*., 2000) to neutralize the deleterious effects at the effector site or interacts with macrophage and increases $CD8^+$ T-cell apoptosis due to caspase-1 activation and IL-18 via increase oxidative stress and MIF (Bagchi and Sinha, 2004; Bagchi *et al*., 2010). Once memory $CD4^+$T-cells are activated by repeated exposure of bacterial antigens either in natural or induced using candidates, leads to development of adaptive immunity by means of generating biased signal

Cellular basis of receptor immune-modulation for mucosal infection has been described schematically (Fig. 1), where immunomodulation of host cell receptors such as TLRs, TCRs, or Cytokine Receptors (CyRs) to *Shigella*-antigen specific signals in order to target vaccines against mucosal infections has been shown. TLRs could be a novel target transport pathway through mucosal cell lining. The focus of our present review is to emphasize the most potential immunomodulators used alone or in combination with adjuvants such as TLR, CyR and TCR ligands, that may confirm high antigenicity following natural and experimental shigellosis. The dearth of mucosal ligands or adjuvants and the mode of its delivery have led researchers to learn about vaccine approaches that only target protective immuno-dominant antigens specific to cellular receptors such as PRRs, CyRs and TCRs. This approach may enhance the immunogenicity of an antigen to develop precise and biased immune response by switching innate response to antigen-specific adaptive immune response to ensure long lasting protection against subsequent infection. Major antigenic fraction of OMPs identified and elicited an antibody response during natural infection. Some of our studies suggest that the TCR ligand, anti-CD3, used as an immunomodulator supplemented with *Shigella* antigen, may develop memory in Cell-Mediated Immune (CMI)-restored patients and help in gaining antigen specific signals for Th1 in Th0 subsets. Vaccines targeting TLRs, CyRs and TCRs may possibly lead to generation of effective immune cell responses. However, their physiological function to influence helper T-cells is yet to be further ascertained in shigellosis. Indeed, there is a need of an immunomodulator that can promote strikingly biased Th1-mediated antibody response in a vaccine formulation against shigellosis.

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Author's Contributions

Original concept and design: AKB; Material and literature search/collection: AKB, RAB, DKH, FJ, PHP, DRS; Manuscript writing: AKB, RAB, DKH, FJ, PHP, DRS; Final editing: AKB RAB, DKH, FJ, DRS, Schematic figure: RAB, FJ, AKB.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved

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