2. The nature of immune response against cholera

Before going into details of immune response against *V. cholerae*, the major antigens, identified from a large number of studies involving clinical samples and animal experiments, as well as human volunteers, are shown in Fig. 2.

Figure 2: Major antigens of *V. cholerae* are shown schematically. All these antigens are either of cell surface associated or secreted type as shown. The organism also carries two chromosomes, a special feature of the members of the family *Vibrionaceae.*
2.1 Immunity acquired from natural infection

The existence of acquired immunity against the disease cholera has been known since very ancient times. Patients recovering from *V. cholerae* infection are either protected against reinfection with the same *V. cholerae* biotype, or the subsequent episodes are less severe. Around 90% to 100% biotype-specific protection lasting for several years has been demonstrated in the volunteer trials with *V. cholerae* O1 in non-endemic areas and in response to natural infection in endemic zones (Levine et al., 1981; Glass et al., 1982). Since *V. cholerae* is a non-invasive pathogen, it is widely believed that protection against cholera is conferred by secretory IgA (sIgA) within the intestinal lumen (Fig. 3). A majority of Bangladeshi patients convalescing from moderate to severe cholera produced high levels of anti-CT as well as anti-LPS sIgA antibodies in the intestine. Elevated sIgA levels were also present in other body fluids, such as breast milk and saliva (Glass et al., 1983). However, sIgA confers only short-term protection, while protective immunity following *V. cholerae* infection is seen in the absence of significantly raised mucosal sIgA. The reason for this long-lasting immunological memory currently remains unknown (Svennerholm et al., 1984a). It is to be noted that the serum titres correlate with protection, but may not be the mechanisms of protection. In addition to clinical disease, repeated natural exposure to *V. cholerae* in the endemic areas may also give rise to significant serum antibody responses (Glass et al., 1985a). Antitoxin antibody levels, however, do not show a similar increase with age. In endemic areas, the age group between two and nine years has the highest risk of developing *V. cholerae* infection, while the younger children may be protected by maternal antibodies acquired through breast-feeding (Glass et al., 1983). Mannose-sensitive haemagglutinin (MSHA) (Fig. 2), a type IV pilus of either O1 El Tor or O139 serogroup could be immunogenic, since both convalescing patients and vaccinees develop anti-MSHA antibody responses. However, the titre is significantly lower compared to that of antibacterial and antitoxin antibodies (Svennerholm et al., 1984b). Another type IV pilus, called toxin-coregulated-pilus (TCP) (Fig. 2), which is a polymer of TcpA subunits, is considered to be a major virulence factor of *V. cholerae*. However, the protective role of anti-TCP antibody is not clear, and long-term immunological memory against TCP has not been studied. A comparative study of immune responses against *V. cholerae* O1 and O139 has shown that, despite the presence of a polysaccharide capsule in the latter serogroup, comparable systemic vibriocidal and antitoxin antibodies, as well as gut-derived antibody (both IgM and IgA) secreting cells (ASCs) in the peripheral blood (Fig. 3), are generated upon natural infection (Qadri et al., 1997b). Further studies are needed to show whether immunological memories against *V. cholerae* O1 and O139 infections are comparable or not.
Figure 3: Schematic diagram showing gut defences and serum immune responses against *V. cholerae* infection and cholera vaccines (see details in text).

2.2 Intrinsic factors influencing the outcome of exposure

Several innate host factors may influence the outcome of exposure to *V. cholerae*. ABO blood group antigens remain the most well studied intrinsic host factors that determine the susceptibility to cholera (Barua & Paguio, 1977; Levine et al., 1979b; Sircar et al., 1981; Glass et al., 1985b). The O blood group antigen has been reported to confer serotype-specific protection against infection with *V. cholerae* O1 (Barua & Paguio, 1977) and O blood group individuals respond to the live attenuated vaccine CVD103-HgR with higher antibody responses compared to the other blood groups (Lagos et al., 1995). On the other hand, patients with O blood group are at increased risk of suffering from more severe symptoms and fatal outcomes when infected with either O1 or O139 serogroup (Faruque et al., 1994; Harris et al., 2005). Hence it has been hypothesized that *V. cholerae* infection may have selected for the low prevalence of blood group O in the Gangetic Delta region (Glass et al., 1985b). Genetic factors other than blood group antigens probably play a role, since the first-degree relatives of cholera patients in Bangladesh were significantly more likely to develop the disease than less closely related members living in the same household, and this association was independent of blood group (Harris et al., 2005). Nutritional status of the host, like levels of zinc or vitamin A supplementation, and recent infections with other organisms, may also influence the outcome of disease development (Tomkins et al., 1993; Roy et al., 2006; Chowdhury et al., 2008). In the same way, microbiological and environmental factors may influence the outcome of exposure (Colwell, 1996 & 2004). Thus, the household contacts of O1, as opposed to O139 index cases, have a higher likelihood of infection, and markedly higher attack rates are seen in individuals living in households that contain more than one clinical case.
2.3 Antibacterial immunity

2.3.1 Anti-LPS immunity

The LPS (Fig. 2) of *V. cholerae* O1 and O139 are the most consistently described antigens associated with protection against cholera. Serologic studies define three O1 antigens associated with B-cell epitopes (A, B and C). It is generally appreciated that LPS of different serotypes possesses unique B-cell epitopes that are protective (Wang et al., 1998; Villeneuve et al., 2000). Although the exact molecular mechanism of how *V. cholerae* LPS elicits immune response is not clear, it has been shown that *V. cholerae* LPS is a T-cell independent type I (TI-1) antigen and does not require T-cell help for antigen-specific antibody production (Jacobs, 1975). LPS activates B-cells by binding to multiple receptors. The lipid A component of LPS binds Toll-like receptor 4 (TLR4) and other TLR-like molecules (Nagai et al., 2002; Peng, 2005). Additionally, complement receptors on B-cells can bind C3d, which may be covalently bound to LPS or to bacterial outer membrane structures. Complement-antigen complex binding to B-cell surface receptors enhances activation by lowering the threshold of cross-linking required (Lyubchenko et al., 2005). In addition, cytokines released from macrophages following LPS binding to their surface TLR4 may modulate B-cell activation (Corbel & Melchers, 1983).

2.3.2 Immunity against non-LPS structural antigens

Systemic and mucosal immune responses against TCP develop in patients infected with *V. cholerae* O1 El Tor and O139 (Fig. 3) and these responses are comparable in magnitude and frequency to those seen with LPS and MSHA (Qadri et al., 1997a; Asaduzzaman et al., 2004). It is to be noted that TCP of classical biotype differs to some extent from that of El Tor (Johnson et al., 1991; Rhine & Taylor, 1994). A volunteer study indicated that the TCP of classical biotype may provide protective immune response in humans (Herrington et al., 1988). Other antigens of *V. cholerae* that are believed to contribute to virulence have also been investigated for induction of antigen-specific immune responses. These include outer membrane proteins (OMPs) like the iron-regulated OMPs, and porin-like proteins that help in colonization of the gut (Sengupta et al., 1992). In particular, an 18 kDa protein that promotes colonization in the infant rabbit model and is present in both biotypes has been suggested as an important protective antigen (Sciortino, 1989). The polar flagellum of *V. cholerae* (Fig. 2) is considered to be a virulence factor and consequently it is believed that anti-flagellar antibodies may exert a protective role by preventing colonization (Yancey et al., 1979; Sinha et al., 1993). However, several non-motile mutants of *V. cholerae* were also evaluated as cholera vaccine, and one such strain called Peru-15 was found to be safe and immunogenic in volunteer trials (Sack et al., 1997; Cohen et al., 2002; Qadri et al., 2005 & 2007). Thus, the role of flageller antigens in immunity against cholera is not clear.
2.4 Antitoxin immunity

CT is one of the most potent oral immunogens ever studied (Lycke & Holmgren, 1986; Williams et al., 1999). Vigorous immune responses are generated, not only against CT administered orally alone, but also against unrelated antigens delivered along with it. Animal studies have shown an indirect correlation between CT-induced fluid secretion and intestinal synthesis of slgA, as well as the number of antitoxin-producing cells in the intestine (Pierce, 1978). Immune responses against CT are mainly directed against the B-subunit (CTB) and neutralization of CT is primarily provided by anti-CTB. Thus, antibodies against CTB are equally effective as antibodies against holotoxin in the protection against CT-induced fluid accumulation in rabbit ileal loops (Svennerholm et al., 1994). CT subunit A (CTA), on the other hand, possesses strong immunomodulatory properties which have a multifactorial basis, including increased antigen uptake by the intestinal epithelial cells (IECs), enhanced antigen presentation by macrophages, isotype switching of IgA in B-cells, increased antigen-specific CD4 T-cell priming and induction of regulatory T-cells (Lycke, 1997), and are mainly contributed by its ADP-ribosylating activity (Lycke, 1997). Moreover, this immunomodulatory role is also observed when CT is co-administered with unrelated protein antigens like keyhole limpet haemocyanin (Snider, 1995; Glenn et al., 1998). Although it is possible to induce biotype-specific monoclonal antibodies against CT, polyclonal antiserum raised against classical CT is equally effective in neutralizing classical and El Tor toxins. It is notable that immunization with classical or El Tor CTB has resulted in equally high titres of antibodies against both these toxins (Kazemi & Finkelstein, 1990).

2.5 Non-specific defense mechanisms

A number of innate defence mechanisms may act as a first line of prevention against colonization of the gut with V. cholerae. Gastric acid milieu is an efficient barrier, and neutralization of the acidity before ingestion of V. cholerae drastically reduces the infectious dose in human volunteers (Cash et al., 1974) (Fig. 3). Cationic antimicrobial peptides that are avidly produced by the mucosal epithelial cells of the gut may also significantly contribute to anti-V. cholerae defence mechanisms (Fig. 3). The bacteria are susceptible to human cathelicidin in vitro while it may evade the host immune response by transcriptionally downregulating cathelicidin expression in the gut mucosa (Islam et al., 2001; Chakraborty et al., 2008). Although V. cholerae was traditionally considered to cause non-inflammatory diarrhoea, several recent studies have reported inflammatory response in the intestine (Silva et al., 1996; Harrison et al., 2008; Qadri et al., 2002 & 2004). Thus, there is neutrophilic infiltration of the gut and increased neutrophils and lactoferrin levels are found in the intestinal lavage fluid. However, the specific role of these neutrophils in the short-term and long-term protection against V. cholerae remains to be elucidated. As mentioned earlier, persons with O blood group are somewhat protected against V. cholerae infection although the mechanism remains unknown.
2.6 Cell-mediated immune response

Considering the non-invasive nature of *V. cholerae* infection, cell-mediated immune responses like the major histocompatibility complex-restricted cellular cytotoxicity, natural killer-cell activity and antibody-directed cell-mediated cytotoxicity (ADCC) probably play a minor role in protective immunity. However, cell-mediated immune response (CMIR) was found to be protective in an infant mouse model (Chaicumpa & Rowley, 1973). Anti-*V. cholerae* immunity may be contributed by increased antigen presentation by different mucosal cells, including enterocytes, as well as enhanced sIgA production (Quiding et al., 1991). Gamma interferon-producing T-cells in the gut mucosa may significantly increase in numbers following antigenic exposure and may play a role in host defence (Quiding et al., 1991). A recent report by Weil et al. (2009) has shown that memory T-cell responses develop at least seven days after *V. cholerae* infection in humans, a time prior to, and concurrent with, development B-cell responses. This result suggests that T-cell responses to *V. cholerae* antigens may be important for the generation and stability of memory B-cell responses. The authors had previously shown that significant numbers of IgG and IgA memory B-cells against protein antigens of *V. cholera*, such as CTB and TcpA, persisted in the gut-associated lymphoid tissue (GALT) after one year of natural infection, while LPS-specific memory B-cells had waned by this time (Harris et al., 2009). As immune response against protein antigens is T-cell dependent, these results further suggest that T-cell help may result in a more durable memory B-cell response to protein antigens (Harris et al., 2009).