ISSN: 03875547
Volume 48, Issue 04, April, 2025

A Review of Immunity Against Extracellular Bacteria: *Vibrio cholera*

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Keywords:
Vibrio cholerae, Th2 immunity, IL-13, humoral response, vaccine development

DOI:
04.11632/Tmj.30.11.2025.01

ABSTRACT

Vibrio cholerae is a Gram-negative bacterium that causes the life-threatening diarrheal disease cholera, which remains a major global health concern despite significant advances in understanding its epidemiology and molecular pathogenesis. The disease predominantly affects populations in developing regions with inadequate sanitation and limited access to clean water. Infection by *V. cholerae* primarily stimulates humoral immune responses, with $CD4^+$ T-helper cell differentiation serving as a central determinant of both disease progression and vaccine efficacy. Among these responses, the Th2 subset and its signature cytokine, interleukin-13 (IL-13), play crucial roles in mucosal defense by inducing goblet cell differentiation, mucus hypersecretion, and antibody production. This coordinated mucosal response facilitates bacterial clearance but can also contribute to excessive fluid loss characteristic of cholera. The balance between Th1, Th2, and Th17 pathways therefore represents a key immunological checkpoint that shapes host protection and the quality of vaccine-induced immunity. This review synthesizes current knowledge on the role of IL-13 and Th2-mediated immunity in *V. cholerae* infection, emphasizing how cytokine regulation and mucosal antibody responses contribute to protection. Furthermore, it explores how these mechanisms can be leveraged to design next-generation cholera vaccines with enhanced and long-lasting immunogenicity. A deeper understanding of IL-13–driven mucosal mechanisms provide critical insight into host–pathogen interactions and offers promising directions for improving both prophylactic and therapeutic strategies against cholera.

1. INTRODUCTION

Vibrio cholerae is a Gram-negative bacterium that causes life-threatening cholera diarrheal disease, transmitted through the consumption of contaminated food or water [1]. Cholera epidemics predominantly occur in developing countries that lack adequate infrastructure for waste management and clean water provision. Seven cholera pandemics have been documented: (1) 1817-1824, originating in Bengal, India; (2) 1829-1837, originating in Russia; (3) 1846-1860 in Russia; (4) 1863-1875 in Bengal, India; (5) 1881-

1896 throughout Europe; (6) 1899-1923 in Russia; and the seventh and most recent pandemic, which originated from Celebes Island, Indonesia, subsequently spreading to Central and South America [2]. The seventh pandemic, which began in 1991, represents the largest and most widespread cholera pandemic, caused by *V. cholerae* biotype El Tor. To date, this disease remains endemic in India and Southeast Asia, where transmission is mediated through water, food, and flies. This bacterium can survive in aquatic environments for approximately three weeks [3]. Given its extracellular nature, understanding the immunological responses—particularly Th2 and humoral pathways—is critical for guiding vaccine development and disease control.

2. VIBRIO CHOLERAE

Vibrio cholerae is a gram-negative bacterium that causes cholera diarrheal disease. Endemic to warmer climates such as Southeast Asia, Latin America, and parts of Africa, *V. cholerae* can be found in aquatic environments including freshwater, saltwater, and brackish water [4], [5]. Approximately 3-5 million people are infected with cholera annually, resulting in an estimated ~140,000 deaths, with half of these fatalities occurring in children aged 5 years or younger [5]. With the current advancement of climate change and rising temperatures, the spread of *V. cholerae* to new geographical regions underscores the urgent need for research advancing our understanding of this pathogen [6], [7].

Table 1. Medically Important Vibrio Species

Organism	Human Disease
Vibrio cholerae serogroup O1 and O139	Epidemic and pandemic cholera
Vibrio cholerae serogroup non-O1/non-O139	Cholera-like diarrhea; mild diarrhea; rarely causes extraintestinal infections
Vibrio parahaemolyticus	Gastroenteritis, wound infections, septicemia
Vibrio vulnificus	Gastroenteritis, wound infections, septicemia

Vibrio cholerae strains capable of causing pandemics and cholera disease are those categorized within serogroups O1 or O139, whereas the majority of *V. cholerae* are environmental "non-O1/O139" strains that may or may not cause some form of gastroenteritis [4]. *V. cholerae* strains causing cholera can be distinguished from one another by their production of cholera toxin (CT) and toxin co-regulated pilus (TCP) [8]. The disease is characterized by symptoms such as profuse watery diarrhea, known as "rice-water stool," which leads to extreme dehydration, shock, and ultimately death [9]. This watery diarrhea is replete with mucus, intestinal epithelial cells, and bacteria. The normally functioning mucus gel layer covering intestinal epithelial cells has numerous functions, including serving as a dynamic defense barrier against both resident and foreign microbes [10]. Secretion of this mucus gel layer is a primary function of a specialized group of cells lining the mucosal tissue, called goblet cells [11].

Alterations in mucin production are hypothesized to occur through several mechanisms: 1) by microbial agent factors that modulate mucin secretion and synthesis, or by altering the chemical composition of these mucins, or 2) host factors released by local epithelial cells or immune cells in response to intestinal microbes [12]. Abundant mucus secretion is one consequence of *V. cholerae* infection and is partly due to the effect of cholera toxin (CT), which triggers massive mucin release through a cyclic adenosine 3′,5′-monophosphate (cAMP) dependent mechanism [13].

Cholera toxin consists of two subunits, CT-A and CT-B, where CT-A comprises subunits CT-A1 and CT-A2. The CT-A1 toxin is the most deleterious subunit as it can increase cAMP levels and cause secretory diarrhea. However, uniquely, this subunit cannot enter intestinal epithelial cells because it cannot bind to

ISSN: 03875547
Volume 48, Issue 04, April, 2025

receptors for cellular entry. The subunit capable of facilitating entry by binding to ganglioside receptors is CT-B. Through CT-B binding to receptors, the CT-AB complex can enter cells via endocytosis, subsequently entering the Golgi apparatus and endoplasmic reticulum to bind with G-proteins as second messengers. G-protein activation increases adenylate cyclase enzyme activity, which converts ATP to cAMP. This cAMP enhances the secretion of Cl^-, HCO_3^-, and H_2O through the cystic fibrosis transmembrane conductance regulator (CFTR) channel. The osmotic pull effect of Cl^- ions attracts Na^+ and H_2O, thus predisposing to dehydration accompanied by electrolyte imbalance [14], [15].

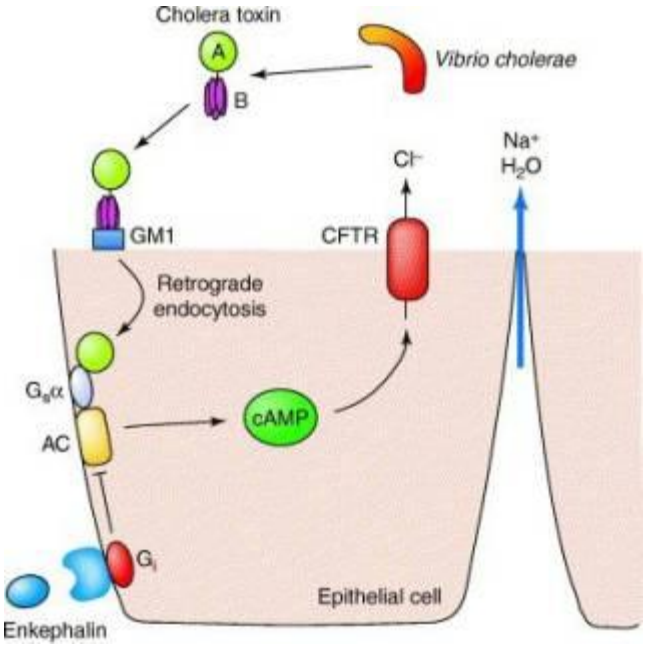

Figure 1. Biomolecular Pathway of Cholera Toxin-Induced Diarrhea [16]

On isolation media, *V. cholerae* appears comma-shaped, curved, and rod-like, measuring 2-4 µm in length. This bacterium is actively motile using a polar flagellum. In prolonged cultivation, vibrios may become straight rods resembling gram-negative bacteria. *V. cholerae* produces convex, smooth, round colonies that appear opaque and granular under transmitted light [17]. *V. cholerae* and most other vibrios grow well at 37°C on various types of media, including certain media containing mineral salts and asparagine as carbon and nitrogen sources. *V. cholerae* grows well on thiosulfate-citrate-bile-sucrose (TCBS) agar, which is a selective medium for vibrios, producing distinctly visible yellow colonies (sucrose fermentation) against the dark green agar background. This indicates an oxidase-positive reaction, distinguishing them from other gram-negative enteric bacteria. Characteristically, vibrios grow at very high pH (8.5-9.5) and are rapidly killed by acid [18]. In areas where cholera is endemic, direct stool culture on selective media such as TCBS and enrichment culture in alkaline peptone water is appropriate. However, routine stool culture on specialized media such as TCBS is generally unnecessary or cost-effective in areas where cholera is rare [19].

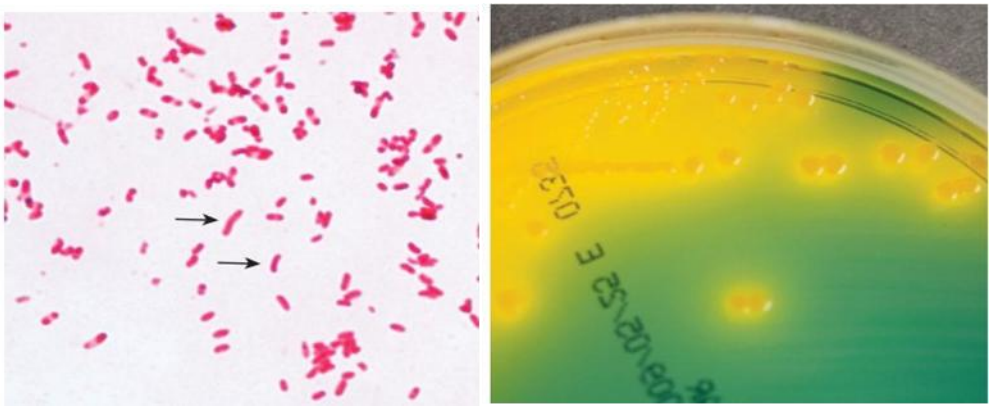

Figure 2. Gram-Negative Comma-Shaped Rods, Motile Due to Polar Flagella (left); Growth of V. cholerae on TCBS Agar [20]

V. cholerae regularly ferments sucrose and mannose but not arabinose. A positive oxidase test result is a key step in the initial identification of *V. cholerae* and other vibrios. Most Vibrio species are halotolerant, and NaCl often stimulates their growth. Some vibrios are halophilic, requiring the presence of NaCl for growth [21].

3. IMMUNE RESPONSE TO VIBRIO CHOLERAE

The influence of *V. cholerae* and cholera toxin on cellular immunity appears to drive the differentiation of CD4+ T cells into Th1 and Th2 cell lineages, with a potential preference toward the Th2 cell type. T cell stimulation by intracellular bacteria generally leads to differentiation toward the Th1 cell lineage and cell-mediated responses, characterized by the activation of phagocytes such as macrophages and cytotoxic T cells (CD8+ T cells), rather than antibody production. Th2 cells, on the other hand, are generally activated by extracellular pathogens and elicit humoral immune responses, characterized by B cell activation and immunoglobulin production [1], [22]. In a report documenting T cell responses in Bangladeshi children, naturally infected cholera patients were shown to have elevated levels of both Th1 and Th2 cells, whereas vaccinated individuals exhibited increased Th1 cells, suggesting that both age and route of immunization may play factors in T cell population dynamics [23]. Meanwhile, [24] demonstrated that oral immunization with CT predominantly induced Th2 cells, with some Th1-type cells still detectable.

One cytokine produced by Th2 cells is IL-13. The primary effect of IL-13 is to induce goblet cell differentiation, resulting in excessive mucus production, which is a consequence of cholera disease [13]. A report by [25] documented elevated levels of this cytokine following stimulation of lymphocytes isolated from hospitalized cholera patients. However, to date, research on Th2 cell production of IL-13 during *V. cholerae* infection remains limited. Another effect of the Th2 cell response is ultimately B cell activation and immunoglobulin production. In humans, *V. cholerae* infection has been shown to induce elevated IgM, IgG, and IgA levels [26], [27].

While extensive research has been conducted to understand cholera immunology and *V. cholerae* pathogenesis, many questions remain in the field. Perhaps the most important question is how to develop a more efficacious cholera vaccine, as current vaccine efficacy is only ~60% [28]. Because Th1 and Th2 responses are antagonistic, with cytokines from one preventing the development of the other, a complete understanding of *V. cholerae* factors that elicit Th1- vs. Th2-mediated responses is therefore critical for improving vaccine efficacy [20]. Our current understanding of CD4+ T cell responses remain incomplete, as does knowledge of the downstream cytokines and effector functions generated by these cells during cholera.

ISSN: 03875547
Volume 48, Issue 04, April, 2025

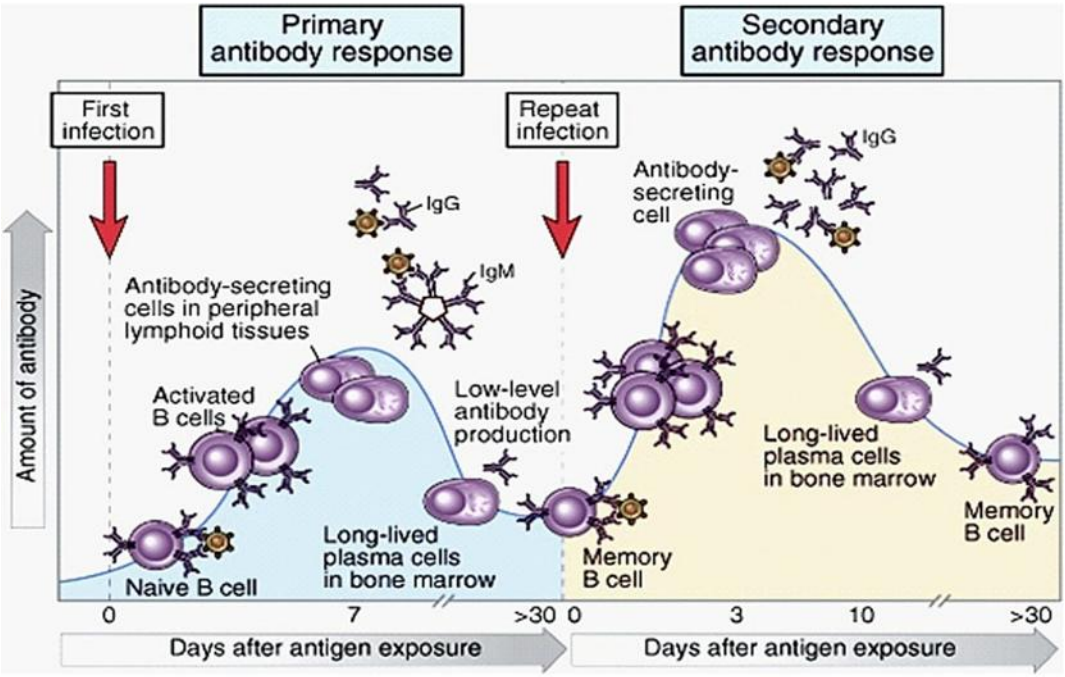

Figure 3. Antibody Formation by B Cells [29]

Previous reports have indicated that there may be roles for both Th1 and Th2 cells in response to V. cholerae infection, while other reports support a more robust Th2 response [23], [24]. It is well documented that Th2 responses generally occur during extracellular pathogen infections. Furthermore, Th2 cells are known as activators of humoral immunity that generate immunoglobulin production, and both mucosal antibodies and IgG have been shown to be present in cholera patients [30]. T-bet and GATA3 act through distal elements to control immune regulatory gene expression. The transcription factor T-bet is essential for Th1 cell-mediated responses, whereas GATA3 is responsible for Th2 cell-mediated immune responses.

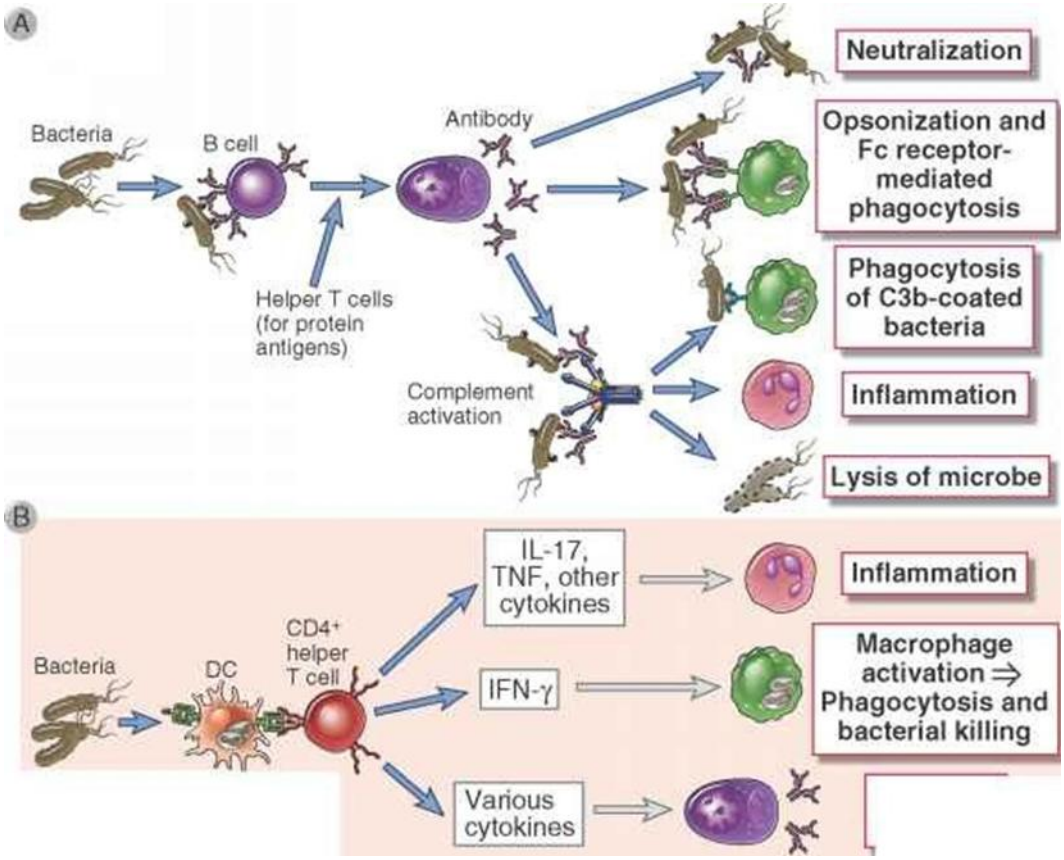

Figure 4. Response Activity Against Extracellular Bacteria [29]

While some evidence in the literature does exist indicating that the Th2 cell lineage is favored in response to cholera, there is far from consensus regarding cell-mediated immunity. Numerous reports of CT serving as an immunomodulatory agent that promotes Th2 cell differentiation are available. A study by [31] demonstrated that in mice, CT modulates dendritic cell (DC) cytokine production to promote Th2 and type 1 regulatory T cells (Tr1), which ultimately inhibits Th1 cell differentiation.

Meanwhile, [32] reported that DCs provide CD4+ T cells independent of Th1-associated cytokines, resulting in substantial generation of Th1, Th2, and Th17 cells. Other reports, such as those by [33], indicate that other *V. cholerae*-associated molecules, such as outer membrane vesicles (OMVs), promote Th2-mediated responses. While the *V. cholerae* strain causing infection and subsequently the virulence factors employed during infection may influence CD4+ differentiation, the data presented here do not demonstrate response differences, as one of the major distinctions between the strains used is the presence of CT.

In humans, immunoglobulins IgM, IgG, and IgA have been shown to increase both during initial *V. cholerae* infection and during secondary challenge [27]. While zebrafish lack IgG, they possess homologs of the important mucosal antibodies IgM and IgA, which play crucial roles in defense against gastrointestinal pathogens such as *V. cholerae* [34]. IgM is known as the first responder antibody; additionally, it has the ability to bind the polymeric immunoglobulin receptor (pIgR), leading to its secretion onto mucosal surfaces such as the intestinal lumen.

Collectively, current evidence indicates that *V. cholerae* infection stimulates a complex interplay of Th1, Th2, and Th17 responses, with a dominant Th2 profile driving antibody-mediated protection and mucosal immunity.

4. CONCLUSION

The immune response to *Vibrio cholerae* is predominantly mediated through humoral mechanisms driven by Th2-type $CD4^+$ T cell activation. Among the cytokines produced, interleukin-13 (IL-13) plays a central role in promoting goblet cell differentiation and mucin hypersecretion, which contribute to both bacterial clearance and the excessive fluid loss characteristic of cholera. The balance between Th1, Th2, and Th17 pathways remains a critical determinant of disease outcome and the quality of protective immunity. Understanding these immune dynamics is essential for developing next-generation cholera vaccines with improved efficacy and longer-lasting mucosal protection. Future research should focus on elucidating how IL-13–driven responses and mucosal antibody production can be modulated to optimize immune protection without exacerbating intestinal pathology. Strengthening our grasp of these mechanisms offers promising avenues for both vaccine innovation and therapeutic strategies targeting *V. cholerae* infection.

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