On Possible Primary Biological Function of Cholera Toxin: Interaction with Vibrio Cholerae Biofilms in the Human Intestine during Infection

Corresponding Author:
Dr. Inessa Z Monastyrskaja,
Researcher, Medical Information Group, Department of Epidemiology, Rostov Research Institute for Plague Control, Gorki str., 117, 344002 - Russian Federation

Submitting Author:
Dr. Inessa Z Monastyrskaja,
Researcher, Medical Information Group, Department of Epidemiology, Rostov Research Institute for Plague Control, Gorki str., 117, 344002 - Russian Federation

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On Possible Primary Biological Function of Cholera Toxin: Interaction with Vibrio Cholerae Biofilms in the Human Intestine during Infection

Author(s): Monastyrskaja IZ

Abstract

A hypothesis is presented that cholera toxin (CT) secreted by toxigenic Vibrio cholerae O1 El Tor binds V. cholerae cells within biofilms formed in the human intestine during infection and interacts with these cells. Interaction of CT with the cells within a biofilm leads to up-regulation of cellular processes and enhances cell growth rate. Positive interbacterial cell regulation is proposed to be the primary biological function of CT. In support of the idea that CT after secretion may interact with V. cholerae cells and enhance their capacity to multiply literary data are presented showing that V. cholerae ctxAB mutants colonize rabbit intestinal mucosa at the level of the toxigenic parent strain when they are co-administrated with CT. The hypothesis about CT interaction with V. cholerae cells in the biofilm formed during cell multiplication in the intestine is supported by the literary data demonstrating that the shared quorum-sensing master regulator in the cell growth state, AphA, turns on simultaneously CT production and biofilm formation. CT as a regulatory enzyme ADP-ribosyltransferase may up-regulate V. cholerae cells and enhance their growth rate.

My opinion

Since its discovery 50 years ago, cholera toxin (CT), a secreted ADP-ribosylating enzyme, has been known as a major virulence factor of toxigenic Vibrio cholerae responsible for cholera diarrhea. However, some literary data suggest that CT may have another function and play a role in the cell regulation in V. cholerae biofilms in the appropriate environment within the intestine. Biofilm formation is a widespread phenomenon critical for bacterial life in the natural environment. Biofilms are formed after bacterial attachment to abiotic and biotic surfaces and are usually described as heterogeneous microbial communities having a compact three-dimensional structure encased in extracellular polysaccharide matrix. It is generally thought that the main function of bacterial biofilms is to protect bacteria from various stresses they encounter in the environment. Biofilm formation is assumed to play a crucial role in the survival of V. cholerae in the aquatic environment for long periods of time. Evidence has been provided that biofilm-mediated attachment to abiotic surfaces may be important for V. cholerae survival in the natural environment [10]. It has been also supposed that as V. cholerae inhabits marine and fresh water, biofilm formation after attachment to the surfaces of aquatic flora and fauna may assist in its survival in the environment during interepidemic periods [1].

Toxigenic V. cholerae O1 El Tor can form biofilms also in the human intestine during infection. Transcriptional regulators VpsR and VpsT positively regulate vps genes involved in the matrix development [1, 8, 12]. The function of biofilms formed in the intestine is unknown. According to current notion, V. cholerae begins to multiply after reaching the intestine, at low cell density (LCD), and multiplication continues till the cell number reaches a certain critical level. Simultaneously with multiplication occurs biofilm formation. Upon reaching the critical level, cell growth instantaneously ceases and bacteria transition into the exit-state leaving the site of multiplication. Biofilm formation is also repressed and a mechanism promoting bacterial detachment from the biofilm formed during multiplication turns on [1, 12]. The level of bacterial growth, transition from one phase to another, biofilm formation, bacterial cell detachment from the biofilm and its dispersal are controlled by the mechanisms of quorum sensing (QS).

At (LCD) QS provides optimal conditions for cell growth. Expression of QS global regulator HapR promoting cell detachment is repressed [1] and bacteria can multiply unhindered. In toxigenic V. cholerae, in contrast to other bacterial species, in which QS activates virulence gene expression at high cell density (HCD), the regulatory cascade leading to production of secreted CT and the CT-coregulated pilus (TCP) is activated at LCD [1], at the beginning of cell multiplication. In this state, in the absence of HapR, regulators AphA and AphB activate tcpPH promoter initiating V. cholerae virulence cascade [12, 7] that includes also ToxR and the downstream regulator, ToxT, that directly activates ctxAB and tcp.
An argument in support of the CT interaction with secreted enzymes may have opposite functions. HA/protease expression suggests also that these two vibrios do not need it any more when multiplication indicates that CT is required only in this phase and production by HapR at the end of cell growth [3, 7]. Repression of the so-called virulence regulon and CT production by HapR at the end of the cell growth ceases and derepressed HapR shuts off the virulence cascade and CT production by binding to aphaA promoter [3]. Thus, when multiplication comes to an end, bacteria do not need CT any more. In the exit-state vibrios use a strategy opposed to that of the cell growth state. Activated HapR directly up-regulates the expression of hapaA which encodes hemagglutinin (HA)/protease, a secreted enzyme that supposedly promotes detachment of cells from biofilms [2].

Recently, Rutherford et al. [7] showed that the transcription factors AphA and HapR are master regulators of QS that operate at LCD and HCD in V. cholerae respectively. By the use of microarray analysis, the authors identified 296 genes whose expression was positively or negatively regulated by AphA in V. harveyi. Gene regulation by V. cholerae AphA was not analyzed but, by analogy, the authors predicted that, at LCD, AphA may regulate many V. cholerae genes. V. cholerae AphA as a master regulator that operates at LCD, that is, in the phase of cell growth must positively regulate genes that are involved in cell multiplication and negatively regulate genes preventing cell growth. However, the best known positive regulatory cascade initiated by AphA is the so-called virulence cascade with its end product, a secreted virulence factor, CT.

The role of CT as a virulence factor that induces cholera diarrhea has been well documented. However, the question why CT is produced in the phase of cell growth when positive effectors of multiplication must be produced has never been addressed. According to logic presented above, namely that only promoting multiplication factors must be positively regulated at LCD, secreted CT may participate in the cell multiplication. CT stimulating cell growth may play a role opposite to that of HA/protease. The main products that bacteria produce in these two distinct phases, cell growth and cell exit, must be involved in achieving their opposite goals. In the exit-phase secreted HA/protease assists in bacterial exit from the site of multiplication for their subsequent dissemination and in order to detach cells from a biofilm the HA/protease must bind these cells. Logically, CT secreted in the phase of cell multiplication may interact with V. cholerae cells stimulating cell growth. Repression of the so-called virulence regulon and CT production by HapR at the end of the cell growth [3, 7] indicates that CT is required only in this phase and vibrios do not need it any more when multiplication stops. The opposing effects of HapR on CT and HA/protease expression suggest also that these two secreted enzymes may have opposite functions. An argument in support of the CT interaction with vibrio cells, that promotes intestinal colonization is provided by the experiments described by Pierce et al. [5]. The authors compared the level of colonization in rabbits using toxigenic V. cholerae strains and nontoxigenic A-B+ or A-B- recombinant mutants derived from them. After oral inoculation, toxigenic strains colonized intestinal mucosa significantly more efficiently than did either A-B- or A-B+ mutants. However, colonization by the mutants was increased to the levels of their toxigenic parent by co-administration of CT with the inoculums. Thus, exogenously added CT, was shown to promote mucosal colonization by V. cholerae and this effect was not due to an interaction of the B subunit with its receptor GM1 because A-B+ mutants colonized no more efficiently than did otherwise isogenic A-B- strains. A similar level of colonization by the toxigenic strains and the mutants administered with CT suggests that the mutants acquired what they have been deprived of, completely restoring their capacity for colonization. The most plausible explanation for these results is that exogenously added CT interacted directly with nontoxigenic mutants changing their properties, including the ability for multiplication, with subsequent increase in the colonization level. Exogenous CT can bind and interact with V. cholerae cells only if CT secreted by the bacteria is capable of doing it and bacteria have a specific ability to interact with secreted CT. Thus, I propose that secreted by V. cholerae CT can interact with vibrio cells changing their properties, and this is the primary biological function of cholera toxin.

Biofilm formation by V. cholerae occurs also at LCD and is continued in the phase of cell growth till the end of this phase [1]. Yang et al. showed recently that indispensible ctxAB gene regulator AphA promotes V. cholerae biofilm development as well, through direct stimulation of the vpsT promoter [11]. Coregulation of CT production and biofilm formation by AphA positive regulator indicates a close link between these two events. The key to understanding the purpose of it may be the findings described by Tamayo et al. [9] though cholera toxin was not referred to in their studies. In the experiments using biofilms of the toxigenic (clinical) V. cholerae O1 El Tor strain C6709, grown in vitro intact and dispersed biofilms colonized the mouse intestine much better than planktonic cells of this strain. The infectious dose for biofilm-derived V. cholerae was substantially lower than that for planktonic cells. The experiments with dispersed biofilms showed that the physiological state and not the biofilm structure was the primary contributor to hyperinfectivity of V. cholerae biofilms and cells derived from them. Biofilm-derived bacteria also
replicated faster than planctonic cells. However, in vitro biofilm-derived and planctonic cells grew equally both in single culture and coculture. The authors suggested that microbes within a biofilm are physiologically distinct from planctonic cells and concluded that growth in a biofilm may prime V. cholerae for enhanced growth upon entry into the human host. In order to reveal a bacterial factor that enhances their physiological state ten mutants of the genes that were shown earlier to be up-regulated in a biofilm were obtained. All mutants colonized the small intestine comparable to the wild type, indicating that the selected effects do not play a role in the hyperinfectivity. All mutants grew equivalently to the wild type in LB broth in vitro.

The findings obtained by Tamayo et al. could be explained with the help of the idea that the secreted CT can interact with V. cholerae cells. If the idea is correct, CT of the toxigenic C6709 strain can be the most suitable candidate for enhancing physiological state and ability to multiply of the cells (or their subpopulation) within a biofilm primed for this stimulatory effect in vitro. Biofilm formation in vivo may enable V. cholerae to determine the level of cell accumulation and establish that the necessary level of the cell growth is reached. When cell accumulation reaches this level controlled by QS spatial constraints within the biofilm may lead to its disruption which may be a signal to the switch to HCD. Then, HapR represses aphA and consequently CT production and biofilm development cease. Stimulation of both, CT production and biofilm formation, by the shared AphA regulator and their simultaneous repression by HapR support this hypothesis. It is well-known that CT is not secreted when V. cholerae is grown in culture media and this may explain why no difference in the growth rate was observed between biofilm-derived and planctonic cells when they were grown in vitro.

If correct, the idea presented here could explain the reason why V. cholerae forms biofilms under the favorable for cell growth conditions in the intestine It also allows to suggest that bacterial biofilms may have more diverse functions than are generally assigned to them, i.e. protection of bacteria from environmental and other stresses.

Since CT role as a major virulence factor causing cholera diarrhea was discovered, the attention of bacteriologists has been directed to revealing mechanisms by which CT induces fluid secretion in the intestine of an infected host. It has been established that CT is a secreted enzyme, ADP-ribosyltransferase. ADP-ribosyltransferases are regulatory enzymes and CT is a positive regulator. CT A subunit possessing ADP-ribosylating activity irreversibly activates cellular adenylate cyclase in the infected host intestine due to the CTA ability to transfer ADP-ribose on stimulatory Gs-alpha. Cellular cAMP accumulation turns on downstream regulatory events which results in massive fluid secretion characteristic of cholera diarrhea.

Activation of bacterial adenylate cyclase and cAMP accumulation as a result of ADPribose transfer, possibly to V. cholerae Glo protein that has a significant sequence similarity to the eukaryotic Gs-alpha [6], may act in the V. cholerae biofilm in the human intestine leading to the up-regulation of cellular processes and faster cell growth. V. cholerae isolated from human stools maintained these properties (“hyperinfectivity”) even after incubation for some hours in pond water [4]. Thus, a biofilm may be the site where the cell up-regulation and faster growth rate occur promoted by cholera toxin.

CT is not a unique bacterial secretory ADP-ribosyltransferase. These enzymes are known to be secreted as toxins by a number of diverse pathogenic bacteria such as enterotoxigenic Escherichia coli, Pseudomonas aeruginosa, Corynebacterium diphtheriae, Clostridium botulinum. If the idea of being an interbacterial regulatory enzyme proves correct for CT, it may be applicable to these secreted ADPribosyltransferases as well. Some other bacterial secreted toxins, for example, adenylate cyclases, are also regulatory enzymes. Thus, their involvement may be supposed as regulators of bacterial processes from without and bacteria may use different systems for secretion of the same extracellular regulators. In the host body, secreted products of pathogenic bacteria interfere with various regulatory pathways and thus are essential for virulence.

References

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