THE ESCHERICHIA COLI/VIBRIO CHOLERAE FAMILY OF ENTEROTOXINS

Randall K. Holmes, Edda M. Twiddy, Carol L. Pickett, Hilda Marcus, Michael G. Jobling, and Francoise M. J. Petitjean

Department of Microbiology
Uniformed Services University of the Health Sciences
Bethesda, Maryland 20814

ABSTRACT

The heat-labile enterotoxins (LT-I and LT-II) of Escherichia coli and cholera enterotoxin (CT) from Vibrio cholerae belong to a family of related protein toxins. Each toxin consists of an A subunit that activates adenylate cyclase in target cells by ADP ribosylation of the regulatory protein Gs and an oligomeric B subunit that binds to plasma membrane receptors on susceptible target cells. The E. coli/V. cholerae enterotoxin family is divided into serogroups based on neutralization tests. LT-I and CT belong to serogroup I, whereas LT-II belongs to serogroup II. Antigenic variants of CT, LT-I and LT-II are produced by V. cholerae and E. coli strains from natural sources. Genes encoding the A polypeptides for all of these toxins are homologous, and both ADP-ribosyl transferase activity and stimulation of the activity by ADP-ribosylation factor (ARF) are conserved functions of the A subunits. Genes for the B polypeptides of the toxins in serogroup I are also homologous, but they have no significant homology with the B subunit genes of the toxins in serogroup II. Gangliosides to which toxins bind with highest affinity are GM1 for LT-I and CT, GD1b for LT-IIa and GD1a for LT-IIb. This paper presents an overview of the E. coli/V. cholerae enterotoxin family and summarizes recent work from our laboratory on these toxins.

INTRODUCTION

Three different families of enterotoxins are now recognized among the enteric, gram negative bacteria: i.e., the heat-labile enterotoxins (LT), the heat-stable enterotoxins (ST), and the Shiga-like toxins (SLT), which are also called Verotoxins (VT). Evidence that each of these toxins can function as virulence factors is reviewed elsewhere. The E. coli/V. cholerae heat-labile enterotoxin family is the subject of this paper.

Cholera enterotoxin (CT) was the first heat-labile enterotoxin from an enteric gram negative bacterium to be purified and characterized. In experimental animals CT causes secretory responses after intraintestinal injection into ligated ileal loops of adult rabbits, lethal diarrhea after...
intraintestinal inoculation in infant rabbits, and many other biological effects; and in humans CT causes the massive secretory diarrhea which is the principal symptom of Asiatic cholera.\(^\text{17}\) Nontoxinogenic mutants of \textit{V. cholerae} are avirulent or dramatically attenuated both in experimental animals and in volunteers.\(^\text{21,43,57}\) It is well established, therefore, that CT is an important virulence factor for \textit{V. cholerae} and plays a central role in the pathogenesis of cholera.

Although enterotoxins are defined by their biologic activities in the gut of humans or animals, detection of putative enterotoxins is greatly facilitated by using tests that can be performed rapidly and inexpensively in cell cultures or in vitro. Particularly convenient tests for heat-labile enterotoxins use cultured mouse Y1 adrenal tumor cells\(^\text{15}\) or Chinese hamster ovary (CHO) cells,\(^\text{30}\) because the toxins cause changes in morphology of these cells that can be detected by direct microscopic observation. Purification of representative heat-labile enterotoxins and preparation of antisera or monoclonal antibodies against them led to the development of a wide variety of immunoassays,\(^\text{5,6,47-50,90}\) and cloning of representative enterotoxin structural genes provided DNA probes to detect genes for related enterotoxins by DNA-DNA hybridization.\(^\text{54,65,66,74,82}\) A broad arsenal of molecular methods is available, therefore, for analyzing the relationships among members of the \textit{E. coli}/\textit{V. cholerae} family of heat-labile enterotoxins.

Many bacteria associated with diarrheal diseases have been tested for production of enterotoxins. Antigenic relatedness between enterotoxins from different sources is usually established by neutralization tests involving homologous and heterologous combinations of toxins and antitoxins. Such tests provided the first evidence that enterotoxins related to CT were produced by other bacteria (Table 1) as well as \textit{V. cholerae}.\(^\text{17,18,34,35,91}\) Evidence that some enterotoxins are antigenically distinct from CT but, nevertheless, are related both structurally and genetically to CT emerged much later.\(^\text{27,32,42,76,77}\) The \textit{E. coli}/\textit{V. cholerae} enterotoxin family is now divided into two serogroups, defined by cross-neutralization reactions.\(^\text{32,42,76}\) CT and the type I heat-labile enterotoxins (LT-I) of \textit{E. coli} belong to serogroup I, whereas type II heat-labile enterotoxins (LT-II) of \textit{E. coli} belong to serogroup II. Antigenic variants of CT, LT-I and LT-II have also been recognized.\(^\text{2,3,19,25,41,43,52,87}\) Representative bacteria that produce toxins belonging to the \textit{E. coli}/\textit{V. cholerae} enterotoxin family are summarized in Table 1, although not all of the toxins from these bacteria have been characterized in detail. The remaining sections of this paper will be limited to the heat-labile enterotoxins produced by \textit{E. coli} and \textit{V. cholerae}.

**STRUCTURE AND IMMUNOCHEMISTRY**

The heat-labile enterotoxins of \textit{E. coli} and \textit{V. cholerae} are oligomeric proteins consisting of one A polypeptide and five B polypeptides held together by noncovalent bonds.\(^\text{26}\) Each polypeptide is synthesized as a precursor with a signal sequence that is removed during secretion and assembly of the holotoxin.\(^\text{39}\) Table 2 compares some of the major properties of the A and B polypeptides of CT and representatives of the LT-I and LT-II toxin groups.

The CT structural gene is highly conserved among strains of \textit{V. cholerae} representing the different biotypes and serotypes.\(^\text{4,88}\) Minor differences in amino acid sequences between CT from various strains have been demonstrated, however, and some of them affect expression of specific epitopes on CT.\(^\text{19}\) The B subunit of CT is immunodominant, and most of the neutralizing antibodies in polyclonal antiserum against CT are specific for the B subunit.\(^\text{17,49}\) Most epitopes on CT-B appear to be dependent on conformation