The channel-forming toxin aerolysin was identified 25 years ago by Bernheimer and Avigad (1), who gave the protein its name. Aerolysin was purified by Buckley et al. (2), and its structural gene, named aerA, was cloned and sequenced almost simultaneously by two groups (6, 7). Since then more than 50 articles describing the expression, secretion, and properties of aerolysin have appeared, and aerolysin has become one of the best-characterized bacterial channel-forming toxins. Virtually all of the available evidence indicates that aerolysin kills cells by forming discrete channels in their plasma membranes (for a recent review, see reference 8). Although it is certainly cytotoxic, it has not been shown to directly alter cyclic nucleotide levels and therefore does not satisfy the definition of an enterotoxin.

Two articles in Infection and Immunity from the group of Chopra have described the purification and mechanism of action (5), and the role in Aeromonas-mediated infection (9), of a cytotoxic enterotoxin from A. hydrophila. The authors state that their protein (referred to as “Act”) is “an aerolysin-related toxin.” In fact, this protein is aerolysin. Its amino acid sequence is essentially the same as the six other aerolysin sequences (from different Aeromonas strains or species) retrieved by a BLAST search (five of these are referred to as aerolysin and the other as a hemolysin). For example, 416 (97%) of the 427 amino acids in the active, biologically relevant form of the protein described by Chopra’s group (accession no. 2126218) are the same as those in the first aerolysin sequence which was obtained (accession no. 113485), and 6 of the 11 differences are conservative.

All of the properties of the protein described by the Chopra group have previously been described for aerolysin, with one minor apparent exception. In one article (4) it is pointed out that the results of mutagenesis of the cytotoxic enterotoxin do not completely correspond to results reported for aerolysin. However, the authors were not working with purified proteins in this study, nor was there evidence that they had confirmed each of the changes they attempted.

The fact that Chopra et al. do not refer to their protein as aerolysin is not productive. Medline searches using aerolysin as a key word do not locate all of their relevant articles, because aerolysin does not appear in the titles, abstracts, or key words. It is difficult to compare their findings with those of others and to determine if data are being duplicated. This is most recently illustrated in their last Infection and Immunity article, in which they describe the testing in a lethal mouse assay of mutants illustrated in their last article, in which they state “that the authors should use the word aerolysin to describe their protein, as all other groups have done, or they should provide convincing evidence that they are working with a different toxin.”

REFERENCEs

Authors’ Reply
Enterotoxins have been classified as cytotoxic and cytotoxic (10) and cause fluid secretory responses in animals irrespective of their capacity to evoke cyclic nucleotide levels in cells. If the statement of Buckley and Howard is true, then heat-stable enterotoxin (STb) of Escherichia coli should not be referred to as an enterotoxin as it does not alter cyclic nucleotide levels in cells (14), and the same is true for many other enterotoxins (12). In 1992, we presented the amino acid (aa) sequence of a cytotoxic enterotoxin (Act) from Aeromonas hydrophila at the American Society for Microbiology meeting, and the sequence was sent to Dr. Buckley at his request. Within aa residues 449 to 462, there was only a 21 to 36% homology between Act and other two aerolysins (2), and within aa residues 482 to 493, there were marked sequence differences (8% homology) between our Act and aerolysin isolated by Buckley’s group (2, 8). Since then, they have corrected the sequence in this region (aa 482 to 493) compared to their originally published sequence (8), and consequently, it matched our sequence (2, 13).

In our recent papers (4, 15), we provided new information about Act that was not known earlier for aerolysin. This information includes, but is not limited to, cholesterol as one of the receptors for the toxin; the inability of Act, unlike aerolysin (6, 7), to bind to glycoporin; isolation and characterization of naturally occurring toxin-deficient mutants of Aeromonas; and generation of potential regulatory mutants of Aeromonas with altered toxin activity. Consistent with these findings, Buckley
and Howard also indicate in their letter that the results of site-directed mutagenesis of Act do not completely correspond to results reported for aerolysin. We believe that these results on mutagenesis are significant and not “one minor apparent exception” because some of the selected mutated toxin proteins purified thus far exhibited the same biological activities as crude toxin preparations. All of the aa changes were confirmed by sequence analysis (5). We have referred to our toxin as a cytotoxic enterotoxin for a number of years based on the hemolytic, cytotoxic, and enterotoxic nature of this toxin. The aerolysin gene sequenced by Chakraborty’s group from Aeromonas sobria (now A. trota) (9) exhibited 76% homology with that of our act (2) and the aerolysin gene sequenced by Buckley’s group (8). Although Chakraborty et al. (1) generated an aerolysin gene-deficient mutant of A. hydrophila (now A. trota), our studies indicated that Act and the aerolysin isolated by Buckley’s group were not identical to A. trota aerolysin based on differential neutralization by a specific monoclonal antibody (3). Therefore, it was important to delete the act gene from an authentic strain of A. hydrophila, particularly as Kuhn et al. (11) reported that A. hydrophila type HG1/BD-2 may be able to produce diarrhea in humans. In our papers, we have indicated that Act is an aerolysin-like molecule because it differs in some respects from aerolysin. Other investigators have named their toxins “aerolysin” primarily based on DNA sequence analysis. However, our studies are based on molecular, biochemical, and biological properties of the toxin. Currently, we strongly feel that we should refer to our toxin as a cytotoxic enterotoxin; however, we will be careful in our future publications so that other investigators will be able to retrieve our relevant articles in Medline searches using aerolysin as a key word.

REFERENCES

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