Mechanisms of Bacterial Pathogenicity That Involve Production of Calmodulin-Sensitive Adenylate Cyclases

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INTRODUCTION

Since its discovery in 1957 by Sutherland, cyclic adenosine 3’,5’-monophosphate (cAMP) has been shown to play a major role as an intracellular second messenger in animal cells. Hormones, drugs, or toxins which alter intracellular cAMP levels in eucaryotic cells have pronounced effects on the metabolism and function of target cells. A number of bacterial toxins have been identified which elevate cAMP levels in mammalian cells, and it is likely that these toxins contribute to the pathogenicity of the bacteria that produce them. Furthermore, these toxins have been very useful tools in studying cAMP-regulated systems in both intact cells and broken cell preparations.

Several bacterial toxins have been shown to alter concentrations of intracellular cAMP in animal cells. Some of these toxins increase intracellular cAMP levels by catalyzing the transfer of an adenosine diphosphate-ribose moiety from nicotinamide adenine dinucleotide to guanine nucleotide regulatory proteins associated with mammalian adenylate cyclase (7, 31). Cholera toxin, produced by Vibrio cholerae, and heat-labile enterotoxin, produced by Escherichia coli, catalyze the adenosine diphosphate-riboseylation of the α subunit of Gs, the guanosine triphosphate-binding protein that couples stimulatory receptors to adenylate cyclase (2, 31, 32). Islet-activating protein (IAP), also known as pertussis toxin, histamine-sensitizing factor, and lymphocytes-promoting factor, is produced by Bordetella pertussis and catalyzes the adenosine diphosphate-riboseylation of Gs, the guanosine triphosphate-binding protein that couples inhibitory receptors to adenylate cyclase (3, 21). Recently, several acylpeptides isolated from the culture filtrates of Bacillus subtilis have been shown to increase cAMP in cultured mammalian cells by acting as potent inhibitors of mammalian cAMP phosphodiesterases (18).

Two other unique toxins from B. pertussis and Bacillus anthracis possess intrinsic adenylate cyclase activity and are strongly stimulated by the eucaryotic regulatory protein calmodulin. Quite clearly, bacteria have been very versatile in devising mechanisms for elevating cAMP levels in animal cells. Presumably, there have been selective pressures for the evolution of these mechanisms. For example, high levels of intracellular cAMP are toxic for many animal cells; an increase in intracellular cAMP can result in the breakdown and release of cellular energy stores. The toxins may also protect invasive bacteria from a host organism’s phagocytic response. High levels of intracellular cAMP in polymorphonuclear neutrophils have been shown to inhibit several phagocyte-associated processes. This review is limited to the calmodulin-stimulated adenylate cyclases produced by B. pertussis and Bacillus anthracis.

ADENYLATE CYCLASE FROM B. PERTUSSIS

B. pertussis is a small, gram-negative, noninvasive bacillus which is the etiologic agent of whooping cough. The disease is initiated by the invasion of the bacterium among the cilia of the respiratory epithelium. The bacterium produces a number of factors which are thought to play a role in pathogenesis (28, 30, 34, 46). One of these factors is a calmodulin-sensitive adenylate cyclase which was first detected in crude vaccine preparations obtained from B. pertussis (52). In addition, adenylate cyclase activity has been detected in several related bacterial strains, Bordetella parapertussis and Bordetella bronchiseptica (6). A recent study has characterized calmodulin-sensitive adenylate cyclase activity associated with a 68,000-molecular-weight protein purified from the outer membrane of B. bronchiseptica (33).

These calmodulin-sensitive bacterial adenylate cyclases are the only adenylate cyclases released extracellularly. Other bacterial adenylate cyclases are primarily membrane associated, like the E. coli adenylate cyclase, or cytoplasmic, as are the enzymes from Brevibacterium liquefaciens or Staphylococcus salinus (20). The B. pertussis enzyme is soluble. It is released into the culture media of bacteria, and it reaches peak activity during the exponential growth phase (6, 13, 16, 28). Release of adenylate cyclase into the culture medium cannot result from cell lysis, because other intracellular enzymes and deoxyribonucleic acid are not released into the media. Hewlett et al. (14) described four distinct compartments with adenylate cyclase activity associated with B. pertussis. The majority of adenylate cyclase activity is associated with either the periplasmic space or the extracellular surface of the cytoplasmic membrane. A soluble fraction released into the culture medium comprises up to 20% of the total activity. Adenylate cyclase activities in...
these three compartments were sensitive to trypsin and accessible to exogenously added adenosine triphosphate (ATP), which is excluded from bacterial cells (27). Approximately 7 to 9% of the total activity was associated with an intracellular pool of enzyme.

The extracytoplasmic enzyme is heat labile; 90% of the activity is lost after heating at 57°C for 20 min. The activity of the enzyme is dependent on divalent metal cations, and maximal activity occurs in the presence of 5 to 10 mM MgCl2 (15). The B. pertussis adenylate cyclase is not affected by α-keto acids, which have been reported to both stimulate and inhibit other bacterial adenylate cyclases (17, 19). Various amphiphilic molecules, including phosphatidylcholine, phosphatidylserine, and nonionic detergents of the Triton X series have a stimulatory effect on the Vmax of the enzyme with no effect on its Km for ATP (49).

### CALMODULIN REGULATION

In 1980, Wolff et al. (50) discovered that B. pertussis adenylate cyclase is stimulated by calmodulin. Hewlett et al. (16) first observed that the specific activity of the adenylate cyclase was much higher when the bacteria were grown on Bordet-Gengou blood agar rather than in Stainer-Scholte liquid synthetic media. Furthermore, they determined that the enzyme activity in the liquid media could be stimulated by the addition of either rabbit sera or an erythrocyte lysate. It was originally thought that hemoglobin activated the enzyme; however, when erythrocyte lysates were passed over a Sephadex G-100 column, the activator was separated from hemoglobin. It appeared that the activator was proteinaceous in nature, since it was sensitive to both trypsin and α-chymotrypsin (12). The activator of the enzyme was ultimately identified as calmodulin (50). Calmodulin stimulation of the adenylate cyclase associated with whole bacterial cells is approximately 100-fold, while stimulation of the supernatant enzyme is generally less, 3- to 35-fold. The adenylate cyclase associated with the intact organisms appears to be very sensitive to calmodulin, with half-maximal stimulation occurring at 45 nM calmodulin. This sensitivity of the enzyme to calmodulin has been exploited as an assay for calmodulin (8, 9).

Trifluoperazine, a drug that antagonizes the binding of calmodulin to calmodulin-regulated enzymes, and troponin I, a calmodulin-binding protein, both inhibit calmodulin stimulation of B. pertussis adenylate cyclase (10, 50). Troponin C and parvalbumin, proteins homologous to calmodulin, do not activate the bacterial adenylate cyclase (24, 50).

Calmodulin regulation of B. pertussis adenylate cyclase differs from calmodulin stimulation of the mammalian adenylate cyclase in several important respects (Table 1). Ca2+ is an obligatory requirement for calmodulin stimulation of the brain enzyme but not for the bacterial enzyme. The addition of EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid) to the calmodulin-stimulated bacterial enzyme does not readily reverse stimulation of the enzyme (50). In fact, the procaryote adenylate cyclase is stimulated by calmodulin in both the presence and the absence of Ca2+ (10, 24). In the presence of 90 μM Ca2+, the apparent Km for calmodulin is approximately 94 μM. In the presence of ≤1 mM Ca2+, the apparent Km for calmodulin is increased 200-fold to 24 nM. The relatively high affinity of the enzyme for calmodulin even at low Ca2+ concentrations indicates that the enzyme will be activated by calmodulin when it enters animal cells regardless of the free intracellular Ca2+ concentration.

In contrast to the mammalian enzyme, the bacterial calmodulin-sensitive adenylate cyclase is insensitive to guanine nucleotides (15). The two enzymes also differ in their Km for ATP; the brain enzyme has a Km of 0.03 mM (22), whereas the bacterial adenylate cyclase has a Km of 0.4 to 1.0 mM (37).

### PATHOGENICITY AND CELL INVASION

Several lines of evidence suggest that B. pertussis adenylate cyclase is a toxin which contributes to the pathology of whooping cough. The enzyme is released extracellularly and is stimulated by a mammalian regulatory protein which is absent from bacteria. Hewlett et al. (13) determined that adenylate cyclase activity is greatly decreased in degraded strains of B. pertussis. This organism exists in at least four distinct serological phenotypes, designated as phases I, II, III, and IV. The variation from a fresh wild-type isolate (phase I) to a rough or degraded phenotype (phase IV) has been shown to represent a series of losses in antigenic and virulence-associated properties. Phase IV bacteria express significantly less adenylate cyclase activity than bacteria in phase I. When fresh clinical isolates are subjected to multiple passes, a loss of adenylate cyclase activity occurs. B. pertussis grown in synthetic media rich in MgSO4 undergoes a transformation to an avirulent phenotype having both decreased adenylate cyclase and IAP activity (1). Finally, Weiss and co-workers (48) discovered that the extracytoplasmic adenylate cyclase is an obligatory requirement for virulence. These investigators examined a series of transposon Tn5 mutants for virulence. They found that the 50% lethal dose of a hemolysin-deficient strain was 200 times greater than that of the wild-type strain. Also, a mutant deficient in both hemolysin and adenylate cyclase was unable to cause a lethal infection at a dose 10,000 times greater than the wild-type 50% lethal dose. Although mutants deficient only in adenylate cyclase were not obtained, the difference between the hemolysin mutant and the hemolysin-adenylate cyclase mutant is most likely due to the loss in adenylate cyclase activity. Furthermore, these investiga-

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**TABLE 1. Comparison of calmodulin-sensitive adenylate cyclases from bovine brain and B. pertussis**

<table>
<thead>
<tr>
<th>Adenylate cyclase from:</th>
<th>Native mol wt</th>
<th>Catalytic Subunit</th>
<th>Km for ATP</th>
<th>Calcium required for calmodulin stimulation</th>
<th>Subcellar location</th>
<th>Biological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine brain</td>
<td>328,000(± 24,000)a</td>
<td>130,000(± 10,000)b</td>
<td>30 μMc</td>
<td>Yesd</td>
<td>Membrane Soluble</td>
<td>Intracellular switch</td>
</tr>
<tr>
<td>B. pertussis</td>
<td>45,000d</td>
<td>45,000d</td>
<td>2 mMd</td>
<td>Noc</td>
<td>Soluble</td>
<td>Toxin invades animal cells</td>
</tr>
</tbody>
</table>

*a Yeager et al., 1985 (53).
*b A. Minocherhomje, N. F. Flowers, and D. R. Storm, unpublished observations.
*c Keller et al., 1980 (22).
*d Shattuck et al., 1985 (37).
*e Greenlee et al., 1982 (10).
tors isolated avirulent mutants differing from wild type by a single insertion, which suggests the possibility of one genetic region responsible for the expression of the traits associated with the virulent phase (47).

Several studies have reported that various preparations of *B. pertussis* adenylate cyclase activity will increase cAMP levels in animal cells, including human neutrophils (4), human erythrocytes, N1E-115 mouse neuroblastoma cells (38), human lymphocytes, S49 cye- murine lymphoma cells, turkey erythrocytes, and rat oocytes (11). Using crude urea extracts from intact bacteria, Confer and Eaton (4) and Hanski and Farfel (11) demonstrated an increase in intracellular cAMP that was apparently not due to endogenous adenylate cyclase activity. Shattuck and Storm (38), using a more purified IAP-free preparation from bacterial culture supernatant, demonstrated an increase in intracellular cAMP in human erythrocytes which contain little or no endogenous adenylate cyclase activity.

The mechanism for invasion of animal cells by *B. pertussis* adenylate cyclase has not been elucidated, and data obtained with inhibitors of receptor-mediated endocytosis do not clearly rule out this process. Ammonium chloride and chloroquine, inhibitors of receptor-mediated endocytosis, did not inhibit the accumulation of adenylate cyclase activity in human neutrophils (5). In contrast, Hanski and Farfel (11) and Confer et al. (5) demonstrated a Ca<sup>2+</sup>-dependent requirement for cAMP accumulation that was inhibited by EGTA. cAMP accumulation was also inhibited by concanavalin A and dansyl cadaverine. Furthermore, toxins which enter animal cells by receptor-mediated endocytosis generally bind tightly to cell receptors at 4°C and enter cells when warmed to 37°C. *B. pertussis* adenylate cyclase does not exhibit this behavior (11).

Both Confer and Eaton (4) and Hanski and Farfel (11) have presented preliminary evidence that the increase in intracellular cAMP observed when animal cells are incubated with impure preparations of *B. pertussis* adenylate cyclase is caused by the bacterial adenylate cyclase entering animal cells rather than stimulation of a mammalian adenylate cyclase by another bacterial factor. The latter effect cannot be ruled out, however, since the bacterial preparations used were heterogeneous and contained IAP activity. Furthermore, it could be argued that the crude bacterial preparations contain an inhibitor of endogenous phosphodiesterases and that this factor is responsible for the cAMP increases. To determine unambiguously that the adenylate cyclase is capable of entering animal cells, it is necessary to demonstrate that incubation of the purified enzyme with animal cells will elevate intracellular cAMP levels.

**PURIFICATION AND CHARACTERIZATION**

Several partial purifications of *B. pertussis* adenylate cyclase have been reported. Hewlett and Wolff (15) partially purified the *B. pertussis* adenylate cyclase by using diethylaminoethyl chromatography and Sephadex G-200 chromatography. The final specific activity of the enzyme was 142 nmol of cAMP/min per mg, and the molecular weight was reported to be 70,600. Even though this group reported that this enzyme was not stimulated by cAMP (12), they subsequently demonstrated calmodulin sensitivity with similar preparations (49, 51). Hanski and Farfel (11) isolated several peaks of calmodulin-sensitive adenylate cyclase activity from 4 M urea extracts of whole bacteria. Gel filtration of concentrated urea extracts on an Ultrogel ACA 34 column gave two peaks of calmodulin-sensitive adenylate cyclase activity which corresponded to molecular weights of approximately 540,000 and 57,000. The specific activities of these preparations were not reported. The fractions which caused an increase in intracellular cAMP in lymphocytes did not correspond to either of these peaks of adenylate cyclase activity but rather to fractions whose position varied depending on the presence or absence of Ca<sup>2+</sup> in the elution buffer. The disparity between the major peaks of adenylate cyclase activity and the cell-invasive activity is unclear. In the presence of Ca<sup>2+</sup>, the factor causing elevations of intracellular cAMP migrated with an apparent molecular weight of 190,000, while in the presence of chelator the invasive factor shifted to an apparent molecular weight of 320,000. Since Stokes radii and not molecular weights are obtained by gel filtration, these data suggest either a Ca<sup>2+</sup>-dependent shape change in the invasive factor or Ca<sup>2+</sup>-dependent association of subunits. These observations and the Ca<sup>2+</sup> requirement for entry into the cell demonstrated by Confer et al. (5) and Hanski and Farfel (11) suggest that Ca<sup>2+</sup> may interact directly with the enzyme and play an important role in cell entry.

Kessin and Franke (23) have reported 110- and 84-fold purification of high (700,000)- and low (50,000 to 65,000)-molecular-weight forms of the calmodulin-sensitive adenylate cyclase. They reported specific activities of 2.97 and 2.28 μmol/min per mg for the large and small forms. These two forms were isolated from both culture media and urea extracts of intact bacteria. Their purification scheme includes hydrophobic and anion-exchange chromatography followed by gel filtration with an Ultrogel ACA 34 column. Calmodulin activation was 200-fold for the large form and 20-fold for the small form. Calmodulin-sensitive activity could be recovered from both sodium dodecyl sulfate-polycrylamide and isoelectricfocusing gels. The 700,000 form of the enzyme migrated with two peaks of activity on an sodium dodecyl sulfate-polycrylamide gel. The major peak of activity had a molecular weight of approximately 50,000, with a minor peak of activity observed at 65,000. The smaller form of the enzyme from gel filtration chromatography migrated as a single peak of activity with a molecular weight of approximately 50,000. There was not a Coomassie blue-stained protein band associated with adenylate cyclase activity. The data from this study suggest that the minimal molecular weight of the enzyme is 50,000 and that larger forms of the enzyme may be aggregates of the catalytic subunit with itself or another subunit.

Shattuck and co-workers (37) have obtained a significant purification of the soluble *B. pertussis* adenylate cyclase by using the purification protocol summarized in Table 2. The first step in the purification is the removal of bacteria by centrifugation and application of the culture media to QAE-Sephadex. Elution of the enzyme with a linear 0 to 1 M NaCl gradient results in the separation of two peaks of calmodulin-sensitive adenylate cyclase activity, designated peaks I and II. Further purification of peak I adenylate cyclase activity is obtained by the addition of calmodulin to the pooled activity and reaplication to QAE-Sephadex. The addition of calmodulin to the adenylate cyclase activity significantly shifts the elution position of the enzyme on QAE-Sephadex and results in further purification of the enzyme. The specific activity of the calmodulin-shifted adenylate cyclase cannot be determined, however, due to contaminating levels of free calmodulin which elutes at the same position as the enzyme. The excess free calmodulin is removed at a later stage of the purification. To ensure complete removal of IAP from the
preparation, the calmodulin-shifted peak I pool is applied to a fetuin-Sepharose column which absorbs IAP (36) but does not adenylate cyclase. Adenylate cyclase preparations which had been over a fetuin-Sepharose column no longer contain any detectable IAP activity. Excess calmodulin is removed by gel filtration over an Ultrogel AcA 44 column.

This purification protocol results in a 1,600-fold purification of the adenylate cyclase to a final specific activity of 608 μmol of cAMP/min per mg, which is the highest specific activity reported for any adenylate cyclase. The molecular weight of the enzyme determined by sucrose density gradient centrifugation and gel filtration chromatography is 43,400 in the absence of calmodulin and 54,200 in the presence of calmodulin. Assuming a specific activity of 608 μmol/min per mg, a molecular weight of 44,300, and one catalytic site per monomer, the minimum turnover number of the enzyme would be 27,000 per min.

The adenylate cyclase obtained by this purification protocol is an appropriate preparation to determine if the enzyme invades animal cells since the enzyme is extensively purified and contains no detectable IAP activity. Shattuck and Storm (38) have examined the effects of the purified adenylate cyclase on intracellular cAMP levels in erythrocytes. Erythrocytes are an excellent model system for examination of cell entry of the enzyme since they contain little or no endogenous adenylate cyclase activity and possess sufficient levels of ATP to be utilized as a substrate for the adenylate cyclase. Various adenylate cyclase preparations obtained during purification of the B. pertussis adenylate cyclase were incubated with human erythrocytes, and the cells were analyzed for the production of intracellular cAMP (Table 3). Adenylate cyclase activity, which has been purified through the Ultrogel AcA 44 gel filtration column, does not increase intracellular cAMP levels in erythrocytes. Peak I adenylate cyclase activity, which is obtained from the first QAE-Sephadex column and subsequently applied to a fetuin-Sepharose column to remove IAP, does increase the intracellular cAMP levels in erythrocytes to a level of 325 pmol of cAMP per 10^6 cells.

There are two possible reasons why the highly purified adenylate cyclase from the Ultrogel AcA 44 column is unable to increase intracellular cAMP levels in erythrocytes. Since this preparation is more highly purified than peak I adenylate cyclase purified through fetuin-Sepharose and gel chromatography, it is possible that a component which facilitates cell entry of the adenylate cyclase is separated from catalytic activity. Alternatively, the more highly purified enzyme has calmodulin complexed to it, which may inhibit penetration of the enzyme into erythrocytes. Therefore, calmodulin was added to peak I adenylate cyclase which had been passed through fetuin-Sepharose and the calmodulin-enzyme complex was assayed for its effects on cAMP levels of erythrocytes (Table 3). Addition of calmodulin to the enzyme completely inhibits the formation of intracellular cAMP catalyzed by the enzyme, indicating that the enzyme-calmodulin complex does not enter erythrocytes. This increase in cAMP in human erythrocytes contrasts with the results obtained by Hanski and Farfel (11), who did not observe any increase in human erythrocytes with urea extracts obtained from whole bacteria.

The increase in cAMP in various eucaryotic cells with different preparations of bacterial adenylate cyclase is rapid. Using material purified as described above from culture supernatants, Shattuck et al. (37) observed a rapid increase in cAMP with a maximal level obtained after 10 min in both human erythrocytes and cultured neuroblastoma cells. There is no detectable lag phase for cAMP formation with these cells, and increases in cAMP production are detectable within 2 to 3 min after incubation of the enzyme with erythrocytes. The observation that calmodulin completely inhibits intracellular cAMP increases caused by the bacterial enzyme indicates that cAMP is not synthesized extracellularly and then taken up by the cells, since there is equivalent or higher adenylate cyclase activity present extracellularly when calmodulin is complexed to the enzyme. Since the increases in intracellular cAMP occurs in cells which contain no endogenous adenylate cyclase activity, the elevated cAMP is not due to stimulation of a mammalian adenylate cyclase by a bacterial factor. These studies support the hypothesis that the soluble extracytoplasmic calmodulin-sensitive adenylate cyclase from B. pertussis is a toxin which enters animal cells and leads to a rapid increase in intracellular cAMP. The mechanism of cell entry and the possible existence of subunits, however, have yet to be determined.
ADENYLYLATE CYCLASE FROM BACILLUS ANTHRACIS

Bacillus anthracis is the pathogenic bacterium responsible for the once common domestic livestock disease anthrax. It was demonstrated by Smith et al. (39) and by Stanley and Smith (43) that Bacillus anthracis produces a three-component protein exotoxin. The components, protective antigen (PA), edema factor (EF), and lethal factor (LF), have been purified by several research groups. PA is an effective immunogen and is used as the human anthrax vaccine (35). Individual components of the exotoxin are not pathogenic, but coinjection of EF and PA causes edema in rabbits and guinea pigs, and coinjection of LF and PA causes death (40–42, 44, 45). Molnar and Altenbern (29) hypothesized that PA binds to tissue receptors and permits subsequent action of LF or EF; however, no enzymatic activities were demonstrated for either LF or EF (44). Leppa (25) demonstrated that EF contains calmodulin-sensitive adenylate cyclase activity and, with the addition of PA, leads to an increase in cAMP in cultured Chinese hamster ovary cells.

Purification and Characterization

Stanley and Smith (43) reported the first purification for EF by sequential chromatography, using diethylaminoethyl-cellulose with a phosphate elution. This procedure was modified by Leppa (26) to include ammonium sulfate precipitation and hydroxyapatite chromatography to separate PA, LF, and EF. Each of these peaks of activity was further purified by diethylaminoethyl-Trisacryl M chromatography. The major polypeptides in the peak of PA, LF, and EF activities have molecular weights of 85,000, 83,000, and 89,000, respectively, and constitute approximately 90% of the total protein in each peak by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A detailed purification protocol has not been published.

Leppa (25) determined that incubation of EF with Chinese hamster ovary cells in the presence of PA causes a dramatic increase in intracellular cAMP. This effect was dependent on the presence of both toxin components; EF alone did not alter intracellular cAMP levels. The increase in cAMP was rapid with no indication of a lag phase. Calmodulin-dependent adenylate cyclase activity was also shown to be associated with EF (26). In contrast to the B. pertussis enzyme, which exhibits a basal activity without calmodulin present, calmodulin is an obligatory requirement for expression of Bacillus anthracis adenylate cyclase activity (26). Calmodulin activates Bacillus anthracis adenylate cyclase activity in EF over a broad range of concentrations, with half-maximal activation occurring at 2 nM calmodulin in the presence of 50 μM Ca2+ (26). Like the B. pertussis enzyme, calmodulin stimulates Bacillus anthracis adenylate cyclase in the presence of excess chelator.

SUMMARY

The role of these calmodulin-sensitive adenylate cyclases in the virulence and pathogenicity of B. pertussis and Bacillus anthracis has not been established. These toxins could have multiple effects on the pathophysiology of whooping cough and anthrax. A detailed discussion of the pathogenesis and pathology of these diseases and potential relationships to these toxins is beyond the scope of this article. Several experimentally supported lines of evidence connecting the toxins to symptoms of their respective diseases have emerged. Localized edema, a typical symptom of anthrax, may be directly related to adenylate cyclase activity associated with the edema factor purified from Bacillus anthracis (25). The intracellular increase in cAMP caused by this toxin may lead to the edematous condition in a manner analogous to the loss of water in the intestine caused by cholera toxin which also increases intracellular cAMP. Similarly, the catarrhal stage (increased mucoid exudate in the lungs) of whooping cough may be due to the adenylate cyclase toxin. Confer and Eaton (4) demonstrated an intracellular increase in cAMP and a concomitant inhibition of oxidative activity in alveolar macrophages and human neutrophils with an adenylate cyclase-containing urea extract from B. pertussis. This inhibitory effect on macrophage function would certainly protect the bacteria from phagocytosis and perhaps lead to the lyphocytosis-leukocytosis observed in this disease. The toxic effects of the adenylate cyclase from B. pertussis may act synergistically or separately from the effects of IAP, which also effects cAMP metabolism in animal cells (3, 21).

The evidence available to date indicates that the extracytoplasmic adenylate cyclases found in the culture supernatants of B. pertussis and Bacillus anthracis are both toxins which enter some animal cells and that they are activated by intracellular calmodulin. Although substantial purifications of both enzymes have been reported, homogeneous enzymes have not yet been obtained in either case and the quaternary structures of both enzymes require further definition. The mechanisms for entry of the enzymes into animal cells remain to be established, and it is not clear whether or not the two enzymes invade animal cells by the same or by distinct mechanisms. Further progress in defining the molecular mechanisms for cell entry of the bacterial adenylate cyclase will depend upon the success in obtaining homogeneous enzyme preparations and the possible identification of other protein subunits which facilitate cell entry.

LITERATURE CITED