http://www.expertreviews.org/

expert reviews in molecular medicine

Immune modulation by the cholera-like enterotoxins

Robert J. Salmond, Jeffrey A. Luross and Neil A. Williams

The role of cholera toxin and heat-labile enterotoxin in the pathogenesis of diarrhoeal disease has been well documented for many years. In addition to these deleterious effects, a wealth of data is accumulating that suggests that these toxins and their subunits might be used to modulate immune responses in a variety of beneficial ways. In this regard, the toxins can boost immune responses to unrelated antigens, leading to the possibility of their use in the generation of improved vaccines to a variety of pathogens. Furthermore, recent evidence suggests that recombinant preparations of the nontoxic B subunits of the toxins have distinct immunomodulatory activities, with potential applications to the treatment of autoimmune and inflammatory diseases. This article reviews our current understanding of the mechanisms of immune modulation by these fascinating proteins.

Cholera toxin (Ctx) from *Vibrio cholerae* and the related heat-labile enterotoxin (Etx) from enterotoxigenic strains of *Escherichia coli* are the causative agents of cholera and travellers' diarrhoea, respectively (Ref. 1). Both toxins are oligomeric proteins, composed of a single A subunit (CtxA/EtxA) and five identical B subunits (CtxB/EtxB), and share approximately 80% identity at the nucleotide level. The crystal structures of Ctx (Ref. 2) and Etx (Ref. 3) indicate that the B subunits are arranged in a symmetrical pentameric ring into which the A

Robert J. Salmond
Postdoctoral Researcher, The Babraham Institute, Laboratory of Lymphocyte Signalling and Development, Babraham, Cambridge, CB2 4AT, UK. Tel: +44 (0)1223 496449; Fax: +44 (0)1223 496023; E-mail: robert.salmond@bbsrc.ac.uk

Jeffrey A. Luross
Postdoctoral Fellow, University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK. Tel: +44 (0)117 928 7585; Fax: +44 (0)117 928 7896; E-mail: j.a.luross@bristol.ac.uk

Neil A. Williams (corresponding author)
Reader in Immunology, University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, University Walk, Bristol, BS8 1TD, UK. Tel: +44 (0)117 928 7886; Fax: +44 (0)117 928 7896; E-mail: neil.a.williams@bristol.ac.uk

Williams laboratory website: http://www.bris.ac.uk/pathandmicro/staff/williamsn.htm

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

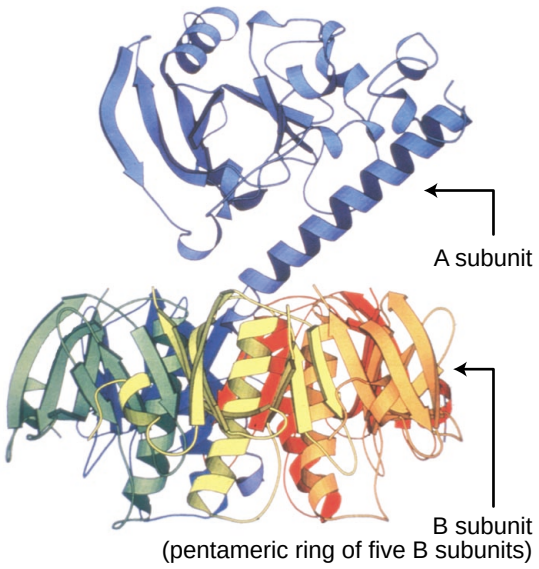
https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

subunit protrudes, forming a highly stable AB_5 structure (Fig. 1). In this regard, both EtxB and CtxB are highly resistant to trypsin and proteinase K degradation, and retain their pentameric quaternary structure down to a pH of 2.0 and 3.9 for EtxB and CtxB, respectively (Ref. 4).

During infection of the gut by *V. cholerae*, Ctx is secreted across the bacterial outer membrane into the extracellular environment (Ref. 5). By contrast, Etx is not actively secreted by enterotoxigenic *E. coli* (ETEC), but is probably released after permeabilisation of the bacterial outer membrane by host bile salts and proteinases (Ref. 6). Etx can also be released via the production of outer membrane vesicles by ETEC (Ref. 7). Following release, the toxins bind to cell-surface receptors via their B subunits. The principal receptor for both CtxB and EtxB is ganglioside G_{M1} [Gal(β1-3)GalNac(β1-4)(NeuAc(α2-3)Gal(β1-4)Glc(β1-1)ceramide] (Ref. 8), a glycosphingolipid found ubiquitously on the surface of mammalian cells. The pentasaccharide moiety of G_{M1} is exposed at the cell surface, anchored into the outer leaflet of the plasma membrane by insertion of the ceramide tail (Fig. 2). Hydrogen bond interactions within each of the five pockets formed by the B subunit pentamer allow CtxB and EtxB to crosslink G_{M1} with extremely high affinity. In addition, both toxins bind ganglioside G_{D1b}, and Etx also interacts with asialo-G_{M1}, lactosylceramide and certain galactoproteins, although with considerably lower affinity (Ref. 9). The receptor-binding activity of the B subunits is required for the uptake and internalisation of the toxins by host cells (Fig. 3), as demonstrated by a complete lack of toxicity when the B subunit has been mutated to disrupt receptor interaction (Ref. 10).

Following trafficking of the toxin to the endoplasmic reticulum, the A subunit enters the cytosol and displays ADP ribosyltransferase activity (Fig. 3). The primary substrate for ADP ribosylation is $G_{S\alpha}$ (Ref. 11), the regulatory subunit of the G protein G_S. Substrate ADP ribosylation results in the activation of adenylate cyclase and an increase in intracellular cAMP concentration. This is followed by chloride secretion, disturbance of membrane osmotic potential and an efflux of fluid from intoxicated cells, resulting in the watery diarrhoea characteristic of infection by *V. cholerae* and ETEC (Ref. 11).

In addition to their roles as mediators of diarrhoeal disease, Ctx and Etx have long been known to be potent mucosal immunogens. Immunisation at mucosal surfaces generally results in a state of immunological tolerance or unresponsiveness to the administered antigen (reviewed in Ref. 12). Ctx and Etx are distinct in that they stimulate strong mucosal immunity both to themselves and to co-administered antigens (Refs 13, 14). The ability of Ctx and Etx to act as adjuvants for mucosal delivery of antigens has made them attractive candidates for vaccine development. Interestingly, it has recently become clear that recombinant preparations of



The crystal structure of *Escherichia coli*: heat-labile enterotoxin

Published in Expert Reviews in Molecular Medicine by Cambridge University Press (2002)

Figure 1. The crystal structure of *Escherichia coli* heat-labile enterotoxin. The B subunit monomers are arranged into a doughnut-shaped pentameric ring (bottom) into which the A subunit is inserted (blue). For further details on the original crystallographic studies, see Ref. 3. The B subunit pentamer is held together by extremely stable noncovalent interactions and is stable at low pH and in the presence of proteinases (Ref. 4). The A subunit, comprising the A_1 and A_2 fragments, forms a wedge-shaped structure. The long α-helix of the A_2 fragment is visible and its C-terminal can be seen inserted into the pore formed within the B subunit. Figure reproduced with permission from Elsevier Science from Ref. 9 **(fig001nwb)**.

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

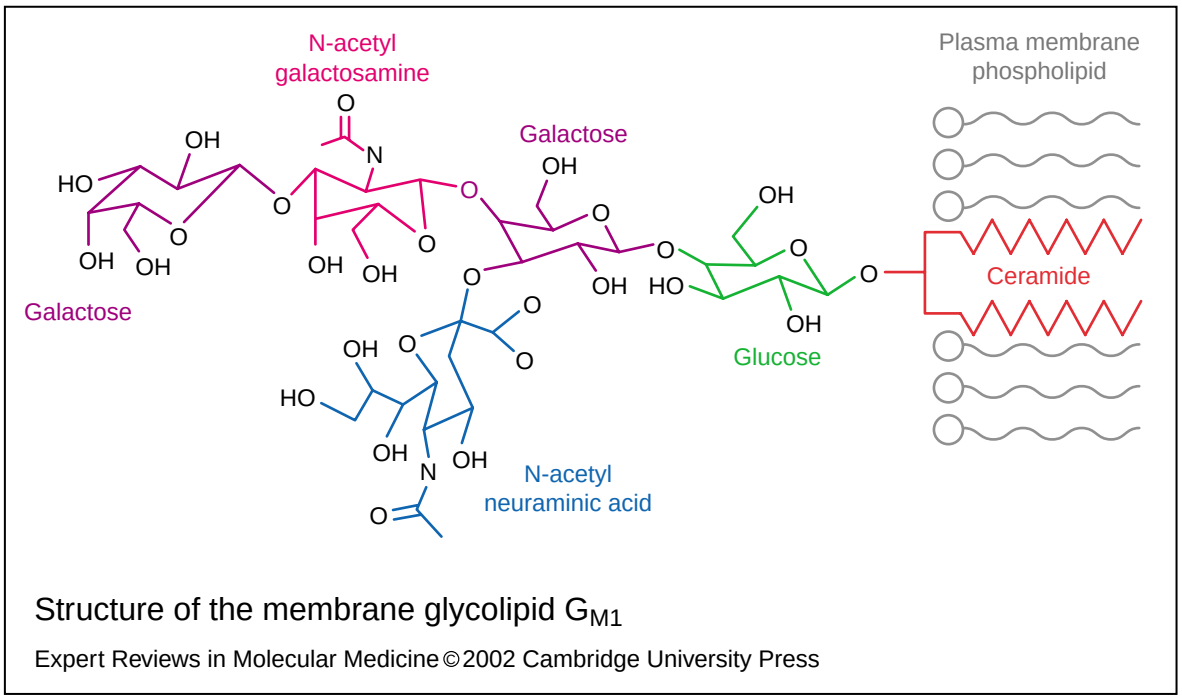


Figure 2. Structure of the membrane glycolipid G_{M1}. The principle receptor for the B subunits of *Escherichia coli* heat-labile enterotoxin (EtxB) and *Vibrio cholerae* toxin (CtxB) is the ubiquitously expressed membrane glycolipid G_{M1} (Refs 8, 9). G_{M1} consists of a pentasaccharide moiety [Gal(β1-3)GalNac(β1-4)(NeuAc(α2-3)Gal(β1-4)Glc(β1-1)] exposed at the surface of the cell membrane and anchored by a ceramide tail. The ceramide tail is inserted into the outer leaflet of the plasma membrane **(fig002nwb)**.

CtxB and EtxB can trigger immune-regulatory processes leading to suppression of inflammatory responses. This review will focus on the mechanisms by which the toxins and their subunits exert these effects.

The adjuvant activity of Ctx and Etx

The first report of the ability of cholera-like enterotoxins to act as adjuvants to unrelated antigens came in 1972 when Northrup and Fauci showed that injection of sheep red blood cells (SRBCs) with Ctx into mice resulted in a 13-fold increase in systemic anti-SRBC antibody titres (Ref. 15). However, interest in their use as adjuvants became much more widely established once it was discovered that they were able to subvert the normal processes of mucosal tolerance, potentiating immunity to co-administered antigens after oral delivery (Refs 13, 14). Subsequent work has highlighted their ability to trigger mucosal and systemic immune responses to a wide variety of antigens.

Despite the high degree of sequence and structural homology between the two toxins, differences in the nature of the immune response stimulated by Ctx and Etx have been reported. Use of Ctx as an adjuvant for the oral administration of keyhole limpet haemocyanin (KLH) resulted in enhanced anti-KLH antibody and T-cell responses, with the production of predominantly T helper 2 (Th2)-associated cytokines (Ref. 16). By contrast, Etx triggers a more balanced cytokine response, involving the production of both Th1 and Th2 cytokines and several antibody classes (Ref. 17).

Nontoxic derivatives

Despite strong supportive data indicating the potential of Ctx and Etx as mucosal adjuvants, their inherent toxicity precludes use as components of human vaccines. As a result, much research has focused on developing nontoxic recombinant derivatives (rCtx, rEtx) that retain these properties. An early report from Lycke and co-workers indicated that mutant rCtx and rEtx that lacked ADP-ribosylating activity failed to act as adjuvants upon oral administration (Ref. 18). This suggested that it might prove impossible to separate toxicity from adjuvant activity.

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

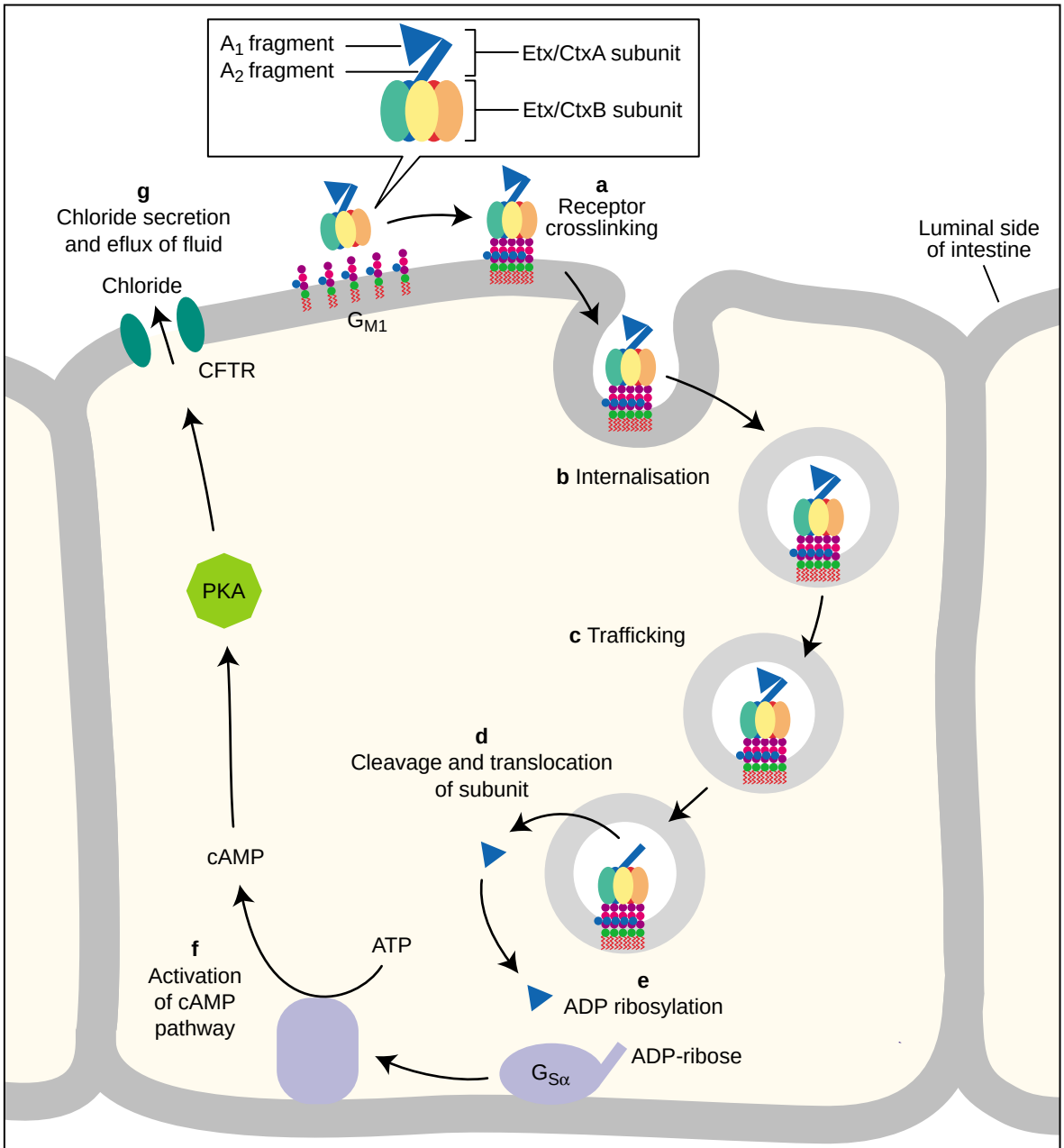


Figure 3. The intoxication process following infection with enterotoxigenic bacteria (*see next page for legend*) **(fig003nwb).**

More recently, several groups have reported that ADP-ribosylating activity contributes to, but is not essential for, the adjuvant properties of Etx and Ctx. For example, a single point mutation in the A subunit of Etx was used to generate LTR72, a protein that retains only 0.6% of the toxicity of the wild-type toxin but that acts as a potent mucosal adjuvant (Ref. 19). Indeed, studies have demonstrated that, at least after intranasal delivery, LTR72 stimulates a stronger mucosal

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

Figure 3. The intoxication process following infection with enterotoxigenic bacteria. The profuse diarrhoea and subsequent fluid and electrolyte loss associated with infection with enterotoxigenic *Escherichia coli* and *Vibrio cholerae* arise following binding of the luminal epithelium of the intestine by the toxins Etx and Ctx, respectively (Refs 1, 11). (a) Binding to the cell is mediated by the ability of the B subunit to interact and crosslink with ganglioside G_{M1} receptors. Crosslinking triggers (b) vesicular uptake and (c) trafficking, a process that is also influenced by the presence of an RDEL (or KDEL in the case of Ctx) sequence on the A_2 fragment (Ref. 67). (d) Enzymatic cleavage allows the A_1 fragment to enter the cytosol, where (e) it catalyses adenosine diphosphate (ADP) ribosylation of $G_{S\alpha}$, a membrane G protein that regulates the activation of adenylate cyclase. (f) Once activated, adenylate cyclase accelerates the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which subsequently induces phosphorylation of protein kinase A (PKA) and leads to the opening of the cystic fibrosis transmembrane regulator (CFTR) chloride ion (Cl^-) channel. (g) Efflux of Cl^- from the cell results in a simultaneous osmotic shift of water from the cell into the intestinal lumen, accompanied by cell death. This results in the watery diarrhoea characteristic of infection by *V. cholerae* and *E. coli* (Ref. 11) **(fig003nwb)**.

response to co-administered antigen than does the wild-type Etx (Ref. 19). However, another mutant of Etx, LTK63, which completely lacks ADP-ribosylating activity, possesses markedly lowered adjuvant activity compared with both wild-type Etx and LTR72. This suggests that the very low level of toxicity of LTR72 can contribute substantially to adjuvanticity (reviewed in Ref. 20) but that it is not a pre-requisite to this activity.

Regardless of the precise role played by the A subunit, it had always been supposed that receptor binding by the B subunit was essential to the ability of the toxins to act as adjuvants. Intriguingly, recent mutational analyses have shown that EtxA alone can act as an adjuvant and that this does not require ADP ribosylation activity (Ref. 21), throwing doubt on this paradigm. A further compounding factor is the route of administration: the mutant toxin described by Lycke and colleagues, LT-E112K (Ref. 18), has since been reported to have the same properties as the wild-type toxin when administered intranasally (Ref. 22). Taken together, these findings suggest that ADP ribosylation is a contributing factor to the adjuvant properties of Etx and Ctx but that it is not essential under conditions of optimal delivery.

Toxin B subunits as adjuvants

Recent work has shown that, when given intranasally at doses of 20 μg, preparations of rEtxB alone can act as adjuvants to herpes simplex virus (HSV) type 1 glycoproteins, inducing a high degree of protective immunity against ocular infection of mice with live virus (Ref. 23). The adjuvant properties of rEtxB were entirely dependent on receptor binding activity as a nonbinding point mutant, EtxB(G33D), failed to induce anti-HSV responses. Protection was associated with the induction of high serum anti-HSV-1 glycoprotein antibody titres and secretory IgA. The immune response was dominated by Th2 cytokines [interleukin 4 (IL-4) and IL-10] and associated antibodies (a high IgG1 to IgG2a serum antibody ratio). Although the immune response generated by immunisation in this way contrasts with that triggered as a result of live virus infection itself (which is strongly Th1 dominated), it is nevertheless likely to be protective for two reasons. First, local antibodies might block virus spread to the peripheral nerves; and, second, the dominant Th2 response protects the eye from the normal processes of Th1-associated immunopathology that are a feature of this disease.

Importantly, rCtxB fails to induce detectable antibody responses to HSV-1 even at doses of up to 100 μg, indicating a clear difference in the properties of the respective B subunits. Millar and colleagues have also reported experiments demonstrating that EtxB is a more potent adjuvant than CtxB (Ref. 24). Such differences might in part be the result of the distinct use of eukaryote cell-surface receptors by EtxB and CtxB (Ref. 9); however, they also might simply reflect differences in the stability of the two molecules (Ref. 4), which could affect the levels of intact B subunit being trafficked across epithelial surfaces to the underlying lymphoid tissues. It has also recently been reported that rEtxB is able to act as an adjuvant to influenza virus haemagglutinin and ovalbumin (Refs 25, 26).

Thus, in seeking to avoid the toxicity of Ctx and Etx, it is now also possible to envisage the use of rEtxB alone as a completely nontoxic alternative to the use of the whole toxins. Its greater inherent stability compared with whole

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

toxin mutants make this an attractive approach. Nevertheless, it is clear that Ctx and Etx and their detoxified mutants trigger stronger antibody responses to co-administered antigens on a dose-for-dose basis when compared with EtxB (e.g. see Refs 26, 27). This indicates that the enzymatic activity and structural properties of the A subunit (Ref. 28), together with the G_{M1}-binding activity of the B subunit (Refs 23, 24, 25, 26), all contribute to adjuvanticity.

EtxB and CtxB as modulators of autoimmune disease

Recent work in several laboratories has shown that CtxB and EtxB can act as immunomodulatory molecules capable of suppressing Th1-associated immune responses. This phenomenon was initially observed by Sun and colleagues, who reported that mice fed CtxB-conjugated SRBCs showed abrogated delayed-type hypersensitivity (DTH) and IgE antibody responses upon systemic immunisation with SRBCs (Ref. 29). The effects on serum IgG and IgM were variable. As expected, neither Ctx nor Etx had this effect; in fact, administration of Ctx and Etx had the reverse effect of priming mice for systemic DTH responses. Importantly, suppression of DTH reactivity by CtxB was shown to be antigen specific as responses to horse red blood cells were unaffected. Subsequently, the ability of CtxB and EtxB to suppress inflammatory responses in an antigen-specific manner has been shown to have potential applications in the treatment of autoimmune disease, as described below.

Experimental autoimmune encephalomyelitis (EAE)

EAE is a $CD4^+$ T-cell-dependent paralytic autoimmune disease of the central nervous system that can be induced by injection of susceptible animals with myelin-derived antigens prepared in complete Freund's adjuvant (CFA). Rats fed CtxB-conjugated myelin basic protein (CtxB–MBP) were protected from induction of EAE (Ref. 30). Furthermore, perhaps more importantly, oral CtxB–MBP was able to protect from ongoing EAE. In a similar study, Bergerot and colleagues reported that CtxB-conjugated insulin protected against spontaneous development of diabetes in the nonobese diabetic (NOD) mouse (Ref. 31). In both systems, protection was associated with a reduction of leukocyte infiltration into the respective sites of inflammation. While no attempt was made to characterise the nature of the immune response in mice treated with CtxB–insulin, analyses of the cellular immune response in the EAE study revealed a clear suppression of IL-2 production and T-cell reactivity to MBP by lymph node cells. This was equivalent to that seen in mice receiving 50–100-fold higher tolerogenic doses of MBP alone.

Unexpectedly, interferon γ (IFN-γ) production by MBP-responding lymph node cells (harvested from the site of MBP/CFA challenge) was elevated in mice receiving low doses of CtxB–MBP. Although counterintuitive to the observed suppression of the T-cell response to MBP and subsequent disease protection (since the disease is known to be driven by Th1 cells, an increase in IFN-γ production by T cells at the site of CFA challenge could be potentially explained by the observations of Matthys and co-workers (Ref. 32). Their data showed that endogenous IFN-γ production is an important component of the concurrent immune response against the mycobacterial component of CFA (Ref. 32). Alternatively, since IFN-γ was the only cytokine assayed, it remains possible that other regulatory cytokines might have been involved in the suppression of MBP-specific T-cell responses.

Collagen-induced arthritis (CIA)

It has been postulated that CtxB is simply acting as a *trans*-epithelial delivery system to facilitate the induction of mucosal tolerance (Ref. 29). In keeping with this, it is widely thought that the conjugation of autoantigen to B subunit is essential for promoting tolerance and the subsequent modulation of autoimmune disease (reviewed in Ref. 9). However, reports have demonstrated that this is not the case, since systemic administration of unconjugated rEtxB or rCtxB can elicit strong protection from autoimmunity. This novel immunomodulatory activity for the B subunits was first described by Williams and co-workers in the CIA model of arthritis using DBA/1 mice (Ref. 33). In these experiments, a single subcutaneous injection of EtxB at the time of disease-inducing challenge resulted in the protection of nearly all mice from developing arthritis. Histological analyses of joints from treated animals revealed a significant reduction in the

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

infiltration of inflammatory cells into the synovium, with no tissue destruction.

EtxB has also been shown to function as a therapeutic in CIA, since treatment 21 days after disease challenge blocked and ameliorated recent-onset CIA (Ref. 33). This immunomodulatory activity of EtxB was shown to be due to receptor binding, since administration of EtxB(G33D), a single point mutant that fails to bind G_{M1}, could not modulate disease. Analysis of T-cell responses to type II collagen (CII) in cultures of draining lymph node cells from EtxB-treated mice revealed a reduction in IFN-γ production, concomitant with an increase in IL-4, implying a shift in the nature of the immune response from Th1 to Th2. In addition, all isotypes of CII-specific IgG immunoglobulins were markedly reduced, with a modest increase in the IgG1 to IgG2a ratio being observed.

More-recent studies have determined that EtxB is also able to treat or prevent CIA following oral or intranasal delivery in the absence of added antigen (Ref. 34). Treatment in this way is associated with a decrease in Th1-associated cytokine production, but has no effect on IL-4 production. The results of this study indicate that mucosal delivery of EtxB might act to enhance the activity of regulatory cells, as shown be the finding that disease protection could be transferred by $CD4^+$ T cells from treated mice (Ref. 34). Depletion of $CD25^+$ cells from this population abrogated the protective effect, while $CD4^+CD25^+$ T cells from treated mice were able to suppress anti-CII IFN-γ production by CII-primed lymph node cells in vitro. The regulatory function of $CD4^+CD25^+$ T cells is thought to play an important role in protection from autoimmune processes in both murine and human systems (reviewed in Ref. 35).

Diabetes

Intranasal rEtxB treatment can block the development of diabetes in the NOD mouse (V. Turcanu and N.A. Williams, unpublished). In this case, treatment is only effective if the delivery of rEtxB is delayed until the anti-islet immune response and per-insulitis have developed. Furthermore, protection from both CIA and diabetes can be transferred with $CD4^+$ T cells from the spleens of treated animals (Ref. 34; V. Turcanu and N.A. Williams, unpublished). Together, these findings indicate that EtxB facilitates the expansion or activation of a population of regulatory T cells. We interpret the finding that abrogation of diabetes in the NOD mouse requires relatively late intervention as indicating that the cell population affected will either return to a resting state or die if it does not enter a site of ongoing inflammation and receive further stimulation. Further analysis is clearly required to investigate this.

Unconjugated rCtxB has also been shown to have immunoregulatory properties that can prevent autoimmune disease. Sobel et al. demonstrated that either intraperitoneal or intravenous administration of rCtxB to NOD mice was highly effective at inducing long-lasting protection from diabetes (Ref. 36). Histological examination of the pancreases of mice receiving CtxB displayed a reduction in the levels of islet destruction. Once again, it was suggested that the islet-sparing activity of CtxB was mediated by a population of regulatory cells, since co-injection of splenocytes from CtxB-treated mice prevented the adoptive transfer of diabetes using splenocytes from diabetogenic animals. However, no attempt was made to characterise the effects of CtxB treatment on the autoimmune response.

Modulation of immune cell function in vitro

In an attempt to understand the mechanisms underlying the immunological properties of Ctx, Etx and their subunits, several studies have focused on the effects of these proteins on purified immune cell populations. Because the main receptor for the toxins is the ubiquitous G_{M1} ganglioside, Ctx/Etx and CtxB/EtxB are able to interact polyclonally with B and T cells, irrespective of their antigen specificity, as well as with antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs).

B and T cells

Both rCtx and rCtxB have been shown to induce isotype differentiation of B cells (Refs 37, 38). In this regard, rCtx acts synergistically with IL-4 to drive isotype switching to the IgG1 antibody subclass (Ref. 37). rCtx was shown to be 1000-fold more efficient at driving isotype differentiation than rCtxB, while this effect could be reproduced using a combination of rCtxB and dibutyryl cAMP. These data suggest that both enzymatic activity and receptor binding contribute to the stimulatory effects of the holotoxin (Ref. 38). In addition, both rCtxB and rEtxB induce B-cell activation, as shown by the upregulation of major

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

histocompatibility complex (MHC) class II and costimulatory molecules on B cells (Refs 39, 40, 41). Collectively, these findings are consistent with the hypothesis that one consequence of receptor binding by the toxins and their B subunits is the lowering of the threshold required for the differentiation of B cells into antibody-secreting cells. It is also possible that B cells acquire an enhanced ability to present antigen to T cells, further promoting the immune response.

In contrast to the stimulatory effects on B cells and professional APCs, both rEtxB (Refs 40, 42) and rCtxB (Ref. 43) induce programmed cell death (apoptosis) of $CD8^+$ T cells. Depletion of $CD8^+$ T cells by rCtxB has also been shown to occur in vivo (Ref. 44). By contrast, the $CD4^+$ T-cell subset is relatively insensitive to rEtxB- and rCtxB-induced apoptosis. The ability of rEtxB to induce apoptosis of $CD8^+$ T cells, as well as to activate B cells and monocytes, is entirely dependent on receptor-binding activity, as is the case for adjuvanticity and treatment of autoimmune disease (Refs 23, 33, 34, 40, 42, 45).

Monocytes and macrophages

Studies have shown that rEtxB triggers production of IL-10, IL-6 and tumour necrosis factor α (TNF-α) by human monocytes (Ref. 45). By contrast, EtxB treatment inhibited secretion of IL-12 by monocytes (Ref. 45), a property that is also mediated by whole Ctx but not rCtxB (Ref. 46). When EtxB-treated monocytes were used as APCs in an allogeneic mixed lymphocyte reaction, IL-10 and IFN-γ production were increased in comparison with levels seen in cultures stimulated with untreated monocytes, indicating that presentation by EtxB-treated monocytes leads to altered T-cell differentiation (Ref. 45). Similarly, Ctx enhances the APC function of macrophages in vitro (Refs 47, 48). Ctx treatment of macrophages results in enhanced MHC class II-restricted presentation of a peptide from hen egg lysozyme to a T-cell hybridoma and a corresponding increase in T-cell proliferation (Ref. 48). Further studies indicate that Ctx enhances expression of the costimulatory molecule B7.2 on macrophages. Injection of mice with an anti-B7.2 antibody inhibited both the mucosal immunogenicity and adjuvant properties of Ctx in vivo (Ref. 49). Recent data have shown that Ctx treatment of endotoxin-stimulated macrophages increases production of IL-10, IL-6 and IL-β, and decreases production of IL-12, TNF-α and nitric oxide (Ref. 50). These data may partially explain the predilection of Ctx to selectively drive Th2-type responses when used as an adjuvant in vivo.

DCs

Ctx treatment of human DCs induces their maturation as shown by upregulation of expression of MHC class II and costimulatory molecules B7.1 and B7.2 (Ref. 51). Ctx-treated DCs are then able to prime naive T cells in vitro, driving their polarisation towards the Th2 phenotype. Activation of DCs might play an important role in the adjuvant properties of Ctx in vivo. Porgador and colleagues have reported that, following intranasal delivery of Ctx and specific cytotoxic T-cell epitopes to C57BL/6 mice, DCs might be the APC responsible for the induction of immunity to the delivered antigen (Ref. 52). In this regard, depletion of DCs from cell cultures isolated from immunised mice abrogated activation of peptide-specific T-cell responses in vitro.

A model for EtxB-mediated immunomodulation

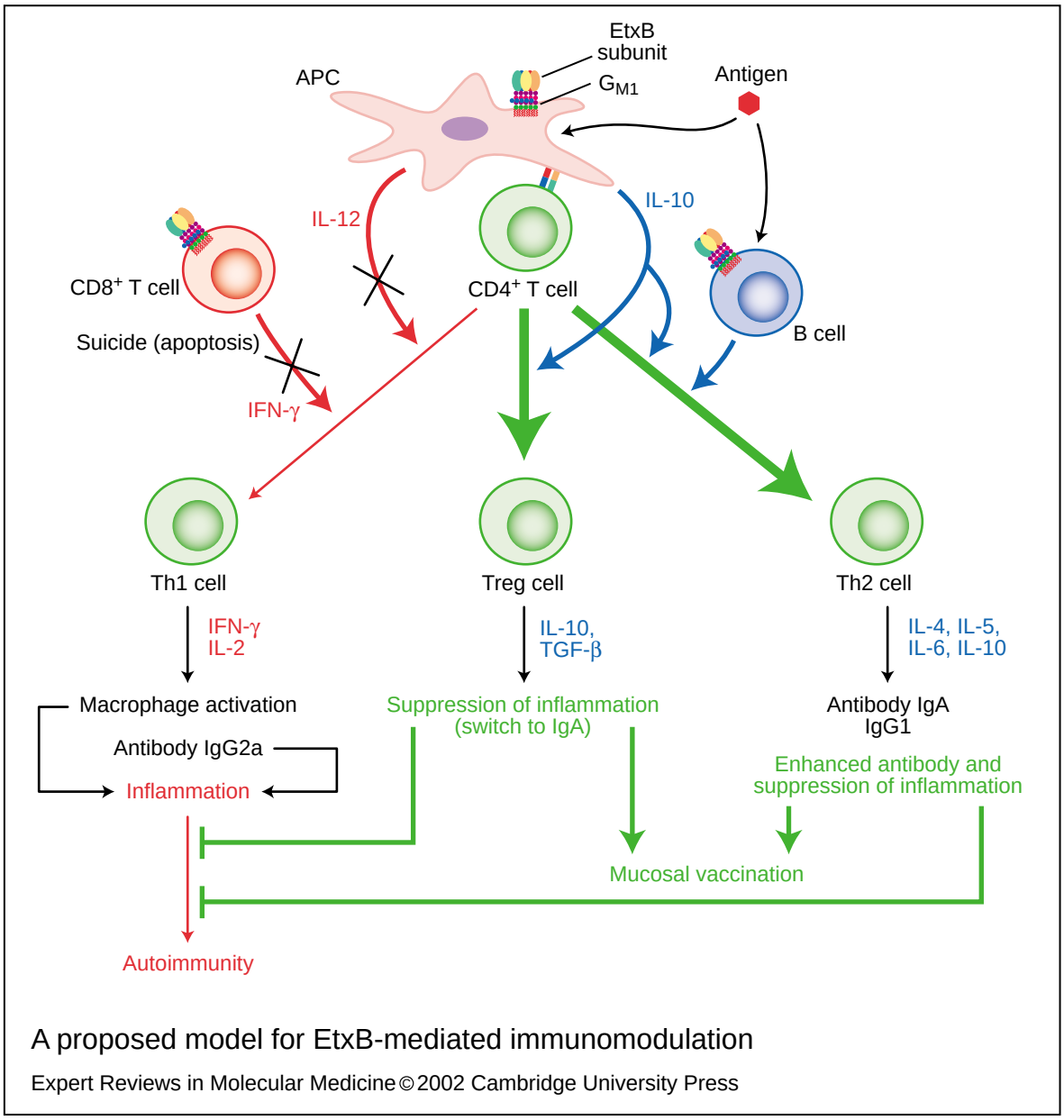
While the relative contributions of B and T cells, monocytes, macrophages and DCs to immunomodulation in vivo are unclear, the above in vitro observations are consistent with the observed alterations to the immune response and enable a model to be proposed to explain how EtxB can act as both an adjuvant and a therapeutic agent in autoimmune disease (Fig. 4). Taken together, the changes to the local microenvironment induced by EtxB would favour the differentiation of T cells away from the Th1 pathway and, instead, favour the generation of T regulatory (Treg)- and Th2-cell populations. Th2 cells would favour the generation of an IgA antibody response, which might be influenced by the presence of transforming growth factor β (TGF-β) from Treg cells and B cells. Both Th2 cells and Treg cells would act to prevent the immunopathological Th1 response in autoimmune disease (Refs 33, 34).

EtxB as a signalling molecule

It is clear that receptor binding by EtxB exerts profound effects upon immune cells both in vivo and in vitro. The complex processes of activation and apoptosis induced by EtxB are likely to involve the triggering of biochemical signalling

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press



A proposed model for EtxB-mediated immunomodulation

Expert Reviews in Molecular Medicine ©2002 Cambridge University Press

Figure 4. A proposed model for EtxB-mediated immunomodulation (*see next page for legend*) (fig004nwb).

pathways in the target cells. In order to begin the process of dissecting out these pathways, recent investigations have focused on why receptor interaction with EtxB induces the apoptosis of $CD8^+$ T cells and activation of B cells in vitro.

Apoptosis: a brief overview

Apoptosis is a genetically controlled process required for embryonic development and regulation of the immune system (reviewed in Ref. 53). For example, cytotoxic $CD8^+$ T cells kill virus-infected host cells by inducing them to undergo apoptosis, thereby limiting viral spread. Furthermore, removal of excess lymphocytes by apoptosis is required for the termination of immune responses.

Unlike necrotic cell death, apoptosis leads to the orderly destruction of cellular structures and the eventual engulfment of the apoptotic cells by phagocytes, thus preventing cell bursting and associated inflammation. In T cells, there are two well-defined biochemical pathways that lead to

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

Figure 4. A proposed model for EtxB-mediated immunomodulation. Interaction of the B subunit of the *Escherichia coli* enterotoxin Etx with its receptor ganglioside G_{M1} modulates the activation of antigen-presenting cells (APCs), T cells and B cells within sites local to delivery. Cytokine production by APCs is modulated, leading to enhanced production of interleukin 10 (IL-10), with a concomitant suppression of IL-12 synthesis. In this way, the major cytokine that drives T helper 1 (Th1) differentiation is blocked in favour of the production of a factor able to promote T regulatory (Treg)-cell differentiation (e.g. see Refs 35, 68). Some local $CD8^+$ T cells undergo apoptosis (Ref. 44) and are thus unavailable as a potential source of interferon γ (IFN-γ) that might otherwise favour Th1 differentiation. B cells are polyclonally activated, a process that might involve them further in antigen presentation and thus favour the induction of Th2 immunity. In addition, this would aid in promoting the response of B cells to admixed antigens and would initiate a stronger antibody response. Taken together, the changes to the local microenvironment induced by EtxB would favour the differentiation of T cells away from the Th1 pathway and, instead, favour the generation of Treg- and Th2-cell populations. Th2 cells would favour the generation of an IgA antibody response, which might be influenced by the presence of transforming growth factor β (TGF-β) from Treg cells and B cells. Both Th2 cells and Treg cells would act to prevent the immunopathological Th1 response in autoimmune disease (Refs 33, 34). In the presence of admixed vaccine antigens, Th2 cells would favour the observed mucosal and IgG1 response, an effect that might be further influenced by certain types of Treg cells **(fig004nwb)**.

apoptosis. In the first, members of the TNF receptor (TNFR) superfamily that possess a cytoplasmic 'death domain' trigger an apoptotic signal upon binding of their respective ligands (Ref. 54). The death-domain-containing members of the TNFR superfamily include the p55 TNFR, p75 TNFR, death receptor 3 (DR3), DR4, DR5 and DR6, and the prototypical death receptor Fas. Upon ligation by Fas ligand (FasL), Fas recruits the adaptor protein Fas-associated death-domain-containing protein (FADD) (Ref. 55). FADD, in turn, recruits the cysteine protease caspase-8, which is then cleaved and activated (Ref. 56). Caspase-8 cleaves and activates several downstream 'effector' proteases such as caspase-3, which in turn cleave a variety of substrates, leading to the breakdown of cellular structures and eventual cell death (Ref. 57).

In the second apoptosis pathway, cellular stresses such as growth factor withdrawal or ultraviolet irradiation induce an apoptotic pathway that is dependent upon mitochondrial damage (Ref. 58). These stimuli lead to activation of pro-apoptotic members of the Bcl-2 family, such as Bax. Bax is thought to form pores in the mitochondrial outer membrane, leading to cytochrome *c* efflux into the cytoplasm (Ref. 59). Cytosolic cytochrome *c* facilitates the activation of an alternative caspase enzyme cascade, via the formation of an 'apoptosome' with the adaptor protein Apaf-1, which recruits and activates caspase-9 in a dATP-dependent manner (Ref. 60). Caspase-9 then cleaves caspase-3 and the cell dies as described above. Caspase-3 activation therefore appears to be a convergent event in the induction of apoptosis by a variety of death stimuli. Activation of apoptosis by the TNFR family can also involve components of the mitochondrial pathway, via the caspase-8-dependent cleavage of the pro-apoptotic Bcl-2 family member Bid (Ref. 61). Caspase-8-mediated cleavage of Bid into tBid facilitates Bax oligomerisation and insertion into the mitochondrial outer membrane (Ref. 62), leading to cytochrome *c* efflux as documented above. The interplay between the Fas and mitochondrial apoptotic pathway is illustrated in Fig. 5a.

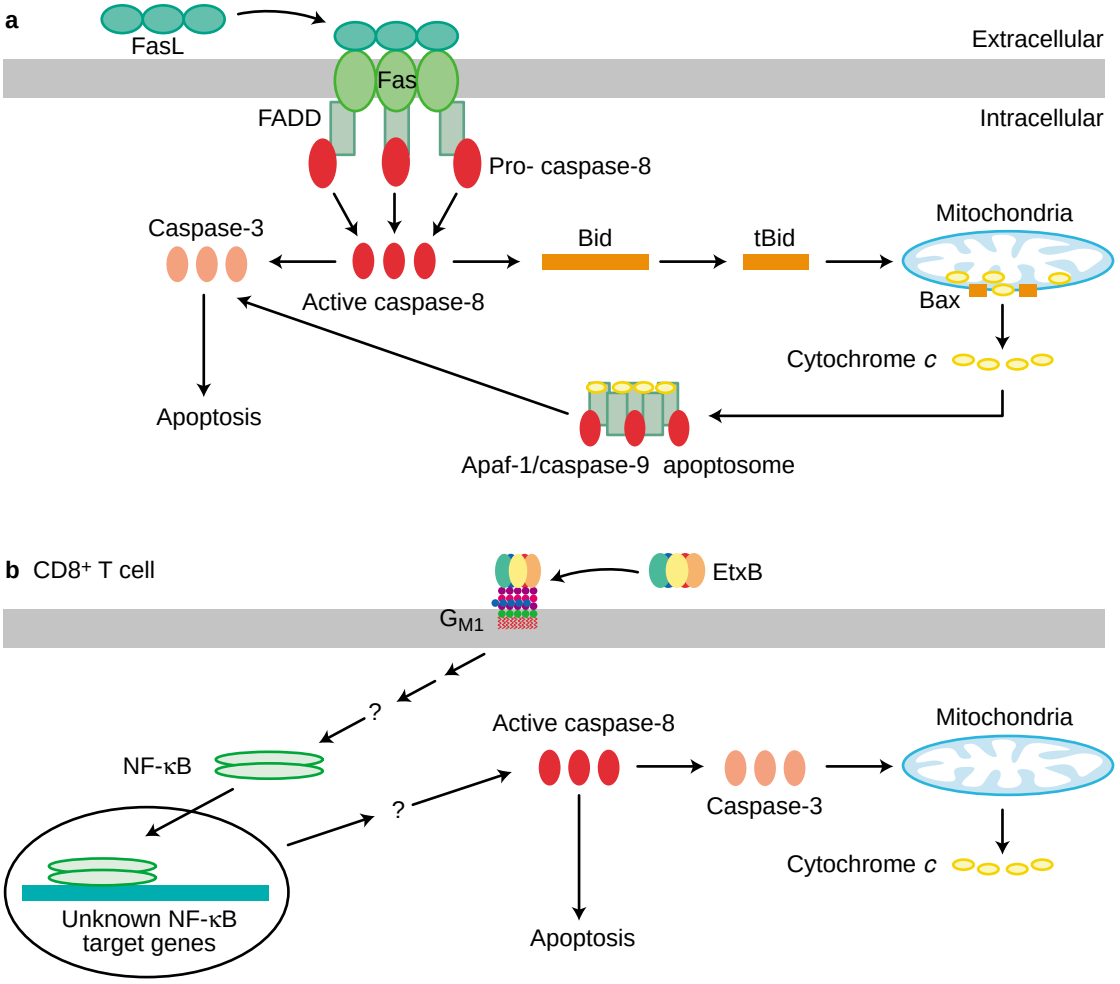
EtxB-mediated apoptosis

EtxB has been shown to induce apoptosis of $CD8^+$ T cells by a novel signalling pathway (Refs 63, 64). EtxB, but not the nonbinding point mutant EtxB(G33D), induces the activation and transcription of caspase-3. Caspase-3 activation was essential to the death process induced by EtxB. Interestingly, rEtxB induced the activation of the transcription factor NF-κB. Experiments using cells from transgenic mice expressing a super-inhibitory form of the physiological NF-κB inhibitor, IκB, indicated that NF-κB activity was critical for apoptosis induction by EtxB (Ref. 63). Furthermore, pre-treatment of cells with a pharmacological NF-κB inhibitor abrogated caspase-3 activation. Despite the absence of enhanced cell death, rEtxB also induced NF-κB activation in the $CD4^+$ T-cell subset. However, activation of NF-κB in $CD8^+$, but not $CD4^+$, T cells was associated with the activation of a downstream target c-Myc (Ref. 64).

It is not clear precisely how NF-κB controls EtxB-induced apoptosis but the process does not require Fas or p55 TNFR, as shown using

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press



Apoptotic pathways induced by Fas and mitochondria, and role of EtxB

Expert Reviews in Molecular Medicine ©2002 Cambridge University Press

Figure 5. Apoptotic pathways induced by Fas and mitochondria, and role of EtxB. (a) Ligation of the Fas receptor by Fas ligand (FasL) leads to recruitment of the death adaptor protein Fas-associated death-domain-containing protein (FADD) (Ref. 55), which in turn recruits and activates pro-caspase-8 (Ref. 55). Active caspase-8 cleaves and activates the effector caspase-3. Caspase-3 functions to cleave downstream substrates including structural components and protective molecules, and activates degradative enzymes, resulting in apoptosis (reviewed in Ref. 57). In addition, caspase-8 cleaves the pro-apoptotic Bcl-2 family member Bid (Ref. 61). The cleaved fragment tBid facilitates Bax oligomerisation and insertion into the mitochondrial outer membrane, leading to cytochrome *c* efflux (Ref. 62). Cytochrome *c* induces the formation of an Apaf-1/caspase-9 'apoptosome' that can activate caspase-3 directly (Ref. 60). (b) Hypothetical model of the death pathway triggered by the B subunit of the *Escherichia coli* enterotoxin Etx in $CD8^+$ T cells. Crosslinking of its receptor ganglioside G_{M1} by EtxB induces the activation of the transcription factor NF-κB by an unknown mechanism (Ref. 63). NF-κB enters the nucleus and regulates the transcription of target genes (e.g. c-Myc) (Ref. 64) and other genes upstream of caspase-8 activation. Caspase-8 cleaves and activates downstream effector caspases, including caspase-3, which then facilitate cell death. Cytochrome *c* efflux occurs downstream of prior caspase and NF-κB activation **(fig005nwb)**.

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

mice defective in these signalling pathways (Ref. 63). However, caspase-8 is activated after EtxB treatment and its inhibition prevents the death process, indicating that it plays a critical role. By contrast, cytochrome *c* efflux is a late event in EtxB-induced cell death and requires the prior activation of NF-κB and caspases. Further investigation is required to define which NF-κB-regulated genes are involved in triggering caspase-8 activation by EtxB. In addition, the biochemical processes linking the interaction of EtxB and its receptor to activation of NF-κB have yet to be determined. A hypothetical model of the EtxB-induced death pathway is summarised in Fig. 5b.

Activation of B-cell signalling pathways by EtxB

Further studies have indicated that G_{M1}-binding by rEtxB induces signalling events in murine B cells. EtxB, but not EtxB(G33D), induces activation of the extracellular signal-regulated kinase (Erk) forms of mitogen-activated protein (MAP) kinases (Ref. 65). Importantly, MAP kinases are known to play central roles in many pathways of immune cell signalling (reviewed in Ref. 66). Erk activation was dependent on phosphoinositide 3-kinase (PI3-K) and protein kinase C (PKC) activity, as determined using pharmacological inhibitors. Furthermore, PI3-K activity was also critical for the upregulation of CD25 and MHC class II expression. MAP kinase activity appeared to be dispensable for the upregulation of CD25 expression, suggesting that EtxB stimulates multiple signalling pathways in B cells.

Research in progress and clinical development

It is clear that current understanding of the mechanisms by which the enterotoxins and their B subunits modulate the immune response is incomplete. The proposed models for their action are based largely on in vitro findings of their effects on cells of the immune system. A key challenge in moving this subject area forward will be to determine which of these effects occurs in vivo and what their relative contributions are to modulating the immune response to co-administered vaccine antigens and autoantigens. In the context of vaccination, this probably involves altering the local lymphoid microenvironment where T and B cells are being activated in response to admixed antigens. In autoimmune disease, the key question is how administration of rEtxB intranasally can trigger the activation and/or expansion of populations of regulatory T cells that are then able to suppress inflammation at distant sites. Are these processes the same as those invoked when rCtxB is used as a delivery vehicle for conjugated autoantigen?

Investigations of the biochemical signalling pathways that lead to these effects will enhance our understanding of the actions of the proteins. It is conceivable that the signalling cascades involved will differ between cell types affected by receptor binding. However, the differences in cellular response might also reflect inherent sensitivities to the same signals between cell lineages.

While these important questions are being answered, the process of testing the toxin mutants and B subunits in human trials is continuing. Several pharmaceutical companies are involved in developing Etx-based products as adjuvants and therapeutics. There are limited data on the tolerability of these molecules in humans, and it is highly likely that efficacy trials will take place within the next two years. Such studies should help determine the power of the approaches outlined in this article to provide potential new treatments for human disease.

Acknowledgements and funding

The authors would like to thank Dr David Morgan (Department of Pathology and Microbiology, University of Bristol, UK) and Dr Stephen Thompson (Department of Immunology, Kings College London, UK) for their constructive peer review and comments on this manuscript. Robert Salmond is funded by the Biological and Biotechnology Research Council of Great Britain (BBSRC) and Jeffrey Luross is supported by The Wellcome Trust. Neil Williams is in receipt of a Wellcome Trust Research Leave Fellowship, and the work of the authors' laboratory referred to herein was supported by grants from The Wellcome Trust, BBSRC and Arthritis Research Campaign.

References

1 Spangler, B.D. (1992) Structure and function of cholera toxin and the related Escherichia coli heat-labile enterotoxin. Microbiol Rev 56, 622-647, PubMed ID: 93125438

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

2 Zhang, R.G. et al. (1995) The three-dimensional crystal structure of cholera toxin. J Mol Biol 251, 563-573, PubMed ID: 95387395
3 Sixma, T.K. et al. (1993) Refined structure of Escherichia coli heat-labile enterotoxin, a close relative of cholera toxin. J Mol Biol 230, 890-918, PubMed ID: 93240541
4 Ruddock, L.W. et al. (1995) Kinetics of acid-mediated disassembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Molecular basis of pH stability. J Biol Chem 270, 29953-29958, PubMed ID: 96102052
5 Hirst, T.R. et al. (1984) Mechanism of toxin secretion by Vibrio cholerae investigated in strains harboring plasmids that encode heat-labile enterotoxins of Escherichia coli. Proc Natl Acad Sci U S A 81, 7752-7756, PubMed ID: 85088485
6 Hunt, P.D. and Hardy, S.J. (1991) Heat-labile enterotoxin can be released from Escherichia coli cells by host intestinal factors. Infect Immun 59, 168-171, PubMed ID: 91099956
7 Horstman, A.L. and Kuehn, M.J. (2000) Enterotoxigenic Escherichia coli secretes active heat-labile enterotoxin via outer membrane vesicles. J Biol Chem 275, 12489-12496, PubMed ID: 20239889
8 Sattler, J. et al. (1978) Studies of ligand binding to cholera toxin, III. Cooperativity of oligosaccharide binding. Hoppe Seylers Z Physiol Chem 359, 719-723, PubMed ID: 78216711
9 Williams, N.A., Hirst, T.R. and Nashar, T.O. (1999) Immune modulation by the cholera-like enterotoxins: from adjuvant to therapeutic. Immunol Today 20, 95-101, PubMed ID: 99198402
10 Guidry, J.J. et al. (1997) Role of receptor binding in toxicity, immunogenicity, and adjuvanticity of Escherichia coli heat-labile enterotoxin. Infect Immun 65, 4943-4950, PubMed ID: 98053929
11 Cassel, D. and Pfeuffer, T. (1978) Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. Proc Natl Acad Sci U S A 75, 2669-2673, PubMed ID: 78202032
12 Weiner, H.L. (1997) Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunol Today 18, 335-343, PubMed ID: 97381558
13 Elson, C.O. and Ealding, W. (1984) Generalized systemic and mucosal immunity in mice after mucosal stimulation with cholera toxin. J Immunol 132, 2736-2741, PubMed ID: 84213972
14 Clements, J.D., Hartzog, N.M. and Lyon, F.L. (1988) Adjuvant activity of Escherichia coli heat-labile enterotoxin and effect on the induction of oral tolerance in mice to unrelated protein antigens. Vaccine 6, 269-277, PubMed ID: 88337870
15 Northrup, R.S. and Fauci, A.S. (1972) Adjuvant effect of cholera enterotoxin on the immune response of the mouse to sheep red blood cells. J Infect Dis 125, 672-673, PubMed ID: 72214392
16 Wilson, A.D. et al. (1991) The in vitro production of cytokines by mucosal lymphocytes immunized by oral administration of keyhole limpet hemocyanin using cholera toxin as an adjuvant. Eur J Immunol 21, 2333-2339, PubMed ID: 92008137
17 Takahashi, I. et al. (1996) Mechanisms for mucosal immunogenicity and adjuvancy of Escherichia coli labile enterotoxin. J Infect Dis 173, 627-635, PubMed ID: 96196122
18 Lycke, N., Tsuji, T. and Holmgren, J. (1992) The adjuvant effect of Vibrio cholerae and Escherichia coli heat-labile enterotoxins is linked to their ADP-ribosyltransferase activity. Eur J Immunol 22, 2277-2281, PubMed ID: 92387231
19 Giuliani, M.M. et al. (1998) Mucosal adjuvanticity and immunogenicity of LTR72, a novel mutant of Escherichia coli heat-labile enterotoxin with partial knockout of ADP-ribosyltransferase activity. J Exp Med 187, 1123-1132, PubMed ID: 98197168
20 Rappuoli, R. et al. (1999) Structure and mucosal adjuvanticity of cholera and Escherichia coli heat-labile enterotoxins. Immunol Today 20, 493-500, PubMed ID: 20001986
21 de Haan, L. et al. (1998) Mutational analysis of the role of ADP-ribosylation activity and GM1-binding activity in the adjuvant properties of the Escherichia coli heat-labile enterotoxin towards intranasally administered keyhole limpet hemocyanin. Eur J Immunol 28, 1243-1250, PubMed ID: 98225037
22 de Haan, L. et al. (1996) Mutants of the Escherichia coli heat-labile enterotoxin with reduced ADP-ribosylation activity or no activity retain the immunogenic properties of the native holotoxin. Infect Immun 64, 5413-5416, PubMed ID: 97101071
23 Richards, C.M. et al. (2001) Protective mucosal immunity to ocular herpes simplex virus type 1 infection in mice by using Escherichia coli heat-labile enterotoxin B subunit as an adjuvant. J Virol 75, 1664-1671, PubMed ID: 21105201

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

24 Millar, D.G., Hirst, T.R. and Snider, D.P. (2001) Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of cholera toxin. Infect Immun 69, 3476-3482, PubMed ID: 21189275
25 Verweij, W.R. et al. (1998) Mucosal immunoadjuvant activity of recombinant Escherichia coli heat-labile enterotoxin and its B subunit: induction of systemic IgG and secretory IgA responses in mice by intranasal immunization with influenza virus surface antigen. Vaccine 16, 2069-2076, PubMed ID: 99012158
26 Douce, G. et al. (1997) Intranasal immunogenicity and adjuvanticity of site-directed mutant derivatives of cholera toxin. Infect Immun 65, 2821-2828, PubMed ID: 97342758
27 Simmons, C.P. et al. (1999) MHC class I-restricted cytotoxic lymphocyte responses induced by enterotoxin-based mucosal adjuvants. J Immunol 163, 6502-6510, PubMed ID: 20054532
28 De Haan, L. et al. (1999) Mucosal immunogenicity and adjuvant activity of the recombinant A subunit of the Escherichia coli heat-labile enterotoxin. Immunology 97, 706-713, PubMed ID: 99388405
29 Sun, J.B., Holmgren, J. and Czerkinsky, C. (1994) Cholera toxin B subunit: an efficient transmucosal carrier-delivery system for induction of peripheral immunological tolerance. Proc Natl Acad Sci U S A 91, 10795-10799, PubMed ID: 95062151
30 Sun, J.B. et al. (1996) Treatment of experimental autoimmune encephalomyelitis by feeding myelin basic protein conjugated to cholera toxin B subunit. Proc Natl Acad Sci U S A 93, 7196-7201, PubMed ID: 96293499
31 Bergerot, I. et al. (1997) A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. Proc Natl Acad Sci U S A 94, 4610-4614, PubMed ID: 97272272
32 Matthys, P. et al. (1999) Enhanced autoimmune arthritis in IFN-gamma receptor-deficient mice is conditioned by mycobacteria in Freund's adjuvant and by increased expansion of Mac-1+ myeloid cells. J Immunol 163, 3503-3510, PubMed ID: 99406848
33 Williams, N.A. et al. (1997) Prevention of autoimmune disease due to lymphocyte modulation by the B-subunit of Escherichia coli heat-labile enterotoxin. Proc Natl Acad Sci U S A 94, 5290-5295, PubMed ID: 97289759
34 Luross, J.A. et al. (2002) Escherichia coli heat-labile enterotoxin B subunit prevents autoimmune arthritis through induction of regulatory CD4+ T cells. Arthritis Rheum 46, 1671-1682, PubMed ID: 22109154
35 Shevach, E.M. (2000) Regulatory T cells in autoimmmunity. Annu Rev Immunol 18, 423-449, PubMed ID: 20297052
36 Sobel, D.O. et al. (1998) The B-subunit of cholera toxin induces immunoregulatory cells and prevents diabetes in the NOD mouse. Diabetes 47, 186-191, PubMed ID: 98178526
37 Lycke, N., Severinson, E. and Strober, W. (1990) Cholera toxin acts synergistically with IL-4 to promote IgG1 switch differentiation. J Immunol 145, 3316-3324, PubMed ID: 91036975
38 Lycke, N.Y. (1993) Cholera toxin promotes B cell isotype switching by two different mechanisms. cAMP induction augments germ-line Ig H-chain RNA transcripts whereas membrane ganglioside GM1-receptor binding enhances later events in differentiation. J Immunol 150, 4810-4821, PubMed ID: 93267083
39 Francis, M.L. et al. (1992) Cyclic AMP-independent effects of cholera toxin on B cell activation. II. Binding of ganglioside GM1 induces B cell activation. J Immunol 148, 1999-2005, PubMed ID: 92185198
40 Nashar, T.O. et al. (1996) Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: receptor binding is essential and induces differential modulation of lymphocyte subsets. Proc Natl Acad Sci U S A 93, 226-230, PubMed ID: 96133910
41 Nashar, T.O., Hirst, T.R. and Williams, N.A. (1997) Modulation of B-cell activation by the B subunit of Escherichia coli enterotoxin: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1. Immunology 91, 572-578, PubMed ID: 98018503
42 Nahar, T.O., Williams, N.A. and Hirst, T.R. (1996) Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes. Int Immunol 8, 731-736, PubMed ID: 96324796
43 Yankelevich, B. et al. (1996) Differential induction of programmed cell death in CD8+ and CD4+ T cells by the B subunit of cholera toxin. Cell Immunol 168, 229-234, PubMed ID: 96228279
44 Elson, C.O. et al. (1995) Morphologic and functional alterations of mucosal T cells by cholera toxin and its B subunit. J Immunol 154, 1032-1040, PubMed ID: 95123053

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

45 Turcanu, V., Hirst, T.R. and Williams, N.A. (2002) Modulation of human monocytes by Escherichia coli heat-labile enterotoxin B-subunit; altered cytokine production and its functional consequences. Immunology 106, 316-325, PubMed ID: 22095345
46 Braun, M.C. et al. (1999) Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor beta1 and beta2 chain expression. J Exp Med 189, 541-552, PubMed ID: 99126481
47 Bromander, A., Holmgren, J. and Lycke, N. (1991) Cholera toxin stimulates IL-1 production and enhances antigen presentation by macrophages in vitro. J Immunol 146, 2908-2914, PubMed ID: 91201849
48 Matousek, M.P., Nedrud, J.G. and Harding, C.V. (1996) Distinct effects of recombinant cholera toxin B subunit and holotoxin on different stages of class II MHC antigen processing and presentation by macrophages. J Immunol 156, 4137-4145, PubMed ID: 96247628
49 Cong, Y., Weaver, C.T. and Elson, C.O. (1997) The mucosal adjuvanticity of cholera toxin involves enhancement of costimulatory activity by selective up-regulation of B7.2 expression. J Immunol 159, 5301-5308, PubMed ID: 98208248
50 Cong, Y., Oliver, A.O. and Elson, C.O. (2001) Effects of cholera toxin on macrophage production of co-stimulatory cytokines. Eur J Immunol 31, 64-71, PubMed ID: 21099439
51 Gagliardi, M.C. et al. (2000) Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming. Eur J Immunol 30, 2394-2403, PubMed ID: 20400256
52 Porgador, A. et al. (1998) Intranasal immunization with cytotoxic T-lymphocyte epitope peptide and mucosal adjuvant cholera toxin: selective augmentation of peptide-presenting dendritic cells in nasal mucosa-associated lymphoid tissue. Infect Immun 66, 5876-5881, PubMed ID: 99043918
53 Lenardo, M. et al. (1999) Mature T lymphocyte apoptosis-immne regulation in a dynamic and unpredictable antigenic environment. Annu Rev Immunol 17, 221-253, PubMed ID: 99286810
54 Wallach, D. et al. (1999) Tumor necrosis factor receptor and Fas signaling mechanisms. Annu Rev Immunol 17, 331-367, PubMed ID: 99286814
55 Chinnaiyan, A.M. et al. (1995) FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 81, 505-512, PubMed ID: 95277837
56 Boldin, M.P. et al. (1996) Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell 85, 803-815, PubMed ID: 96279826
57 Earnshaw, W.C., Martins, L.M. and Kaufmann, S.H. (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. Annu Rev Biochem 68, 383-424, PubMed ID: 20331259
58 Kroemer, G. and Reed, J.C. (2000) Mitochondrial control of cell death. Nat Med 6, 513-519, PubMed ID: 20264559
59 Jurgensmeier, J.M. et al. (1998) Bax directly induces release of cytochrome c from isolated mitochondria. Proc Natl Acad Sci U S A 95, 4997-5002, PubMed ID: 98226756
60 Li, P. et al. (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91, 479-489, PubMed ID: 98050923
61 Luo, X. et al. (1998) Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94, 481-490, PubMed ID: 98394474
62 Desagher, S. et al. (1999) Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J Cell Biol 144, 891-901, PubMed ID: 99187126
63 Salmond, R.J. et al. (2002) CD8+ T cell apoptosis induced by Escherichia coli heat-labile enterotoxin B subunit occurs via a novel pathway involving NF-kappaB-dependent caspase activation. Eur J Immunol 32, 1737-1747, PubMed ID: 22110064
64 Soriani, M., Williams, N.A. and Hirst, T.R. (2001) Escherichia coli enterotoxin B subunit triggers apoptosis of CD8(+) T cells by activating transcription factor c-myc. Infect Immun 69, 4923-4930, PubMed ID: 21340378
65 Bone, H., Eckholdt, S. and Williams, N.A. (2002) Modulation of B lymphocyte signalling by the B subunit of Escherichia coli heat-labile enterotoxin. Int Immunol 14, 647-658, PubMed ID: 22035445
66 Rincon, M. (2001) MAP-kinase signaling pathways in T cells. Curr Opin Immunol 13, 339-345, PubMed ID: 21299329
67 Lencer, W.I. et al. (1995) Targeting of cholera toxin and Escherichia coli heat labile toxin in polarized epithelia: role of COOH-terminal KDEL. J Cell Biol 131, 951-962, PubMed ID: 96074761

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press

68 Asseman, C. et al. (1999) An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. J Exp Med 190, 995-1004, PubMed ID: 99439780

Further reading, resources and contacts

Moss, J. et al. (eds) (1995) Bacterial Toxins and Virulence Factors in Disease, Marcel Dekker Inc., New York

9th European Workshop on Bacterial Protein Toxins. Abstracts. (2000) Int J Med Microbiol. 290 (Suppl 30), A1-95, PubMed ID: 11305319

Discussion of Safety Evaluation of Toxin Adjuvants, following a meeting at the National Institute of Allergy and Infectious Diseases (NIAID) in 2001 to discuss the safety of proceeding with clinical trials of bacterial toxin adjuvants Ctx and Etx delivered intranasally:

http://www.niaid.nih.gov/dmid/enteric/intranasal.htm

Williams laboratory website:

http://www.bris.ac.uk/pathandmicro/staff/williamsn.htm

Features associated with this article

Figures

Figure 1. The crystal structure of *Escherichia coli* heat-labile enterotoxin (fig001nwb)
Figure 2. Structure of the membrane glycolipid G_{M1} (fig002nwb)
Figure 3. The intoxication process following infection with enterotoxigenic proteins (fig003nwb)
Figure 4. A proposed model for EtxB-mediated immunomodulation (fig004nwb)
Figure 5. Apoptotic pathways induced by Fas and mitochondria, and role of EtxB (fig005nwb)

Citation details for this article

Robert J. Salmond, Jeffrey A. Luross and Neil A. Williams (2002) Immune modulation by the cholera-like enterotoxins. Exp. Rev. Mol. Med. 1 October, http://www.expertreviews.org/02005057h.htm

Accession information: (02)00505-7a.pdf (short code: txt001nwb); 1 October 2002
ISSN 1462-3994 ©2002 Cambridge University Press

https://doi.org/10.1017/S1462399402005057 Published online by Cambridge University Press