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Experimental strategies to combat cytotoxic effects of TcdA and TcdB

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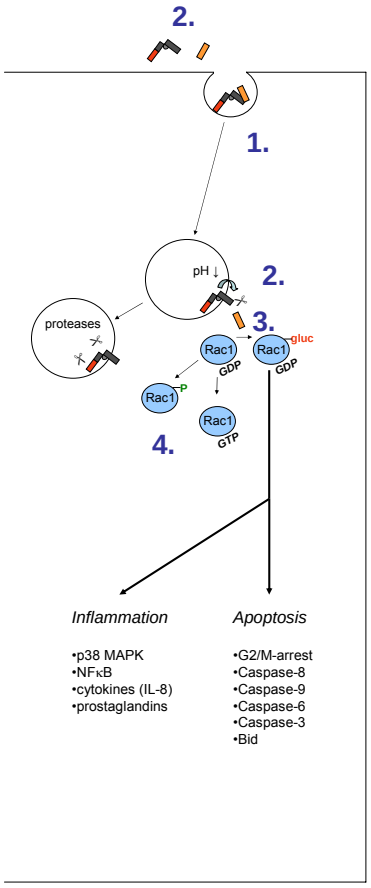
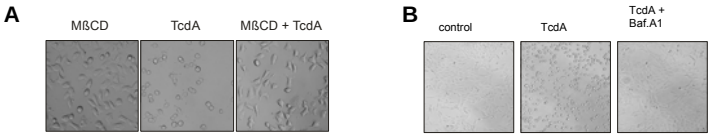
Introduction

To date there are no other specific inhibitors of *C.difficile* toxins A and B than neutralizing antibodies. Antibodies only affect extracellular toxins but not those that already bound to or were endocytosed into host cells. New insights into structure and function of toxins reveal starting points for counteracting features of toxins that are crucial for cytotoxicity.
We here present and evaluate different strategies to reduce or inhibit the cytotoxic effects of TcdA and TcdB

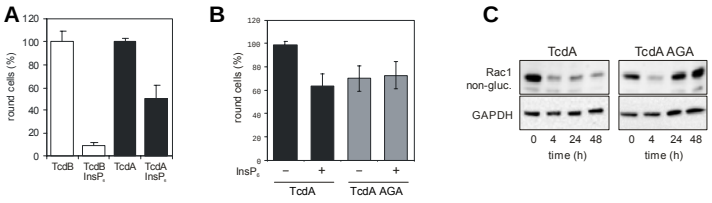


1. Targeting endocytosis and translocation



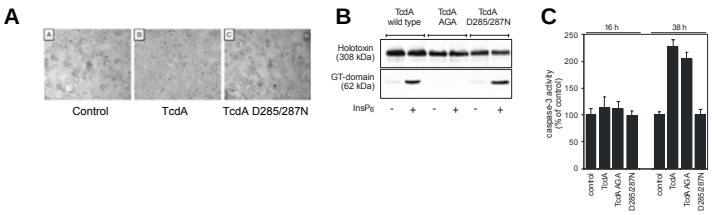
A) Uptake and translocation of TcdA depends on cholesterol. Depletion of cholesterol by methyl-ß-cyclodextrin almost completely inhibits toxin effects as shown here in cell rounding assay.
B) Inhibition of vacuolar H+-ATPase by Bafilomycin A1 prevents translocation of glucosyltransferase domain into cytosol and thereby blocks all toxin effects

2. Targeting autoproteolysis



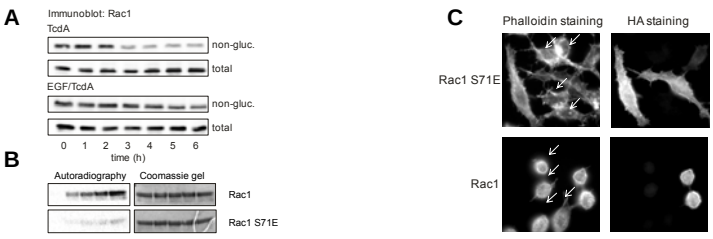
A) Extracellular cleavage of the GT-domain by addition of IP_6 moderately reduces toxicity of TcdA and strongly of TcdB.
B) Inhibition of autoproteolytic cleavage by mutation of cleavage site reduces cytotoxic potency but does not completely inactivate TcdA (see also Fig. 3B).
C) Non-cleavable toxin A (TcdA $A^{541}G^{542}A^{543}$) exhibits only transient effects, since GT-domain does not accumulate within cytosol

3. Targeting glucosyltransferase



A) Specific inhibition of glucosyltransferase of TcdA, realized by site directed mutagenesis of the DXD-motif, resulted in complete inactivation.
B) Mutation of DXD-motif does not affect cysteine protease activity.
C) Inhibition of glucosyltransferase activity but not of autoproteolytic cleavage abrogates cytotoxic effects of TcdA.

4. Targeting substrate GTPases



A) Activation and/or phosphorylation of Rac1 as induced by EGF renders the GTPase in an non- or less glucosylable state, respectively.
B) Phosphomimetic Rac1 is lesser substrate than wild type Rac1 as tested in *in vitro* UDP[^{14}C]-glucosylation assay.
C) Transfection of Hep-2 cells with phosphomimetic Rac1 delays cell rounding induced by TcdA.

Summary

Promising targets for inhibition of TcdA and TcdB: *Glucosyltransferase > translocation > autoproteolysis > substrate GTPases*

Different features of TcdA/TcdB and phases of intoxication process were evaluated regarding their potential to serve as target for inhibition of cytotoxicity. Only two strategies completely abolished all cytotoxic effects of TcdA or TcdB: Inhibition of either translocation or glucosyltransferase activity resulted in a complete block of toxin effects. Although extracellular cleavage of TcdA and TcdB completely inactivates them, quantitative autoproteolysis of toxins can hardly be achieved. Inhibition of autoproteolysis reduces cytotoxicity but does not inactivate the toxins. Since inhibition of cytosolic translocation of toxin domains by inactivating vacuolar proton pump ATPase severely affects host cells, another strategie is favoured. Specific inhibition of the glucosyltransferase should be in focus.