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The Protective Role of Albumin in *Clostridium difficile* Infection: A Step Toward Solving the Puzzle

To the Editor—We read with interest the study by Tabak et al[1] that assessed the predictive risk factors for hospital-onset *Clostridium difficile* infection (CDI) at the time of inpatient admission. According to these authors' findings, patients with a serum albumin ≤3 g/dL had an odds ratio of 2.23 to develop CDI.

These findings add to a body of epidemiological work that implicates low albumin levels as a risk factor for both acquiring and developing severe CDI.[2–5] First, it was thought that hypoalbuminemia was merely a consequence of CDI-induced protein-losing enteropathy.[6] Evidence from subsequent studies showed that hypoalbuminemia actually predisposes the patient to CDI. However, the mechanism by which hypoalbuminemia predisposes the patient to the disease is not yet understood.

Albumin is the most abundant protein in plasma; it has a plethora of properties: it acts as the transporter of several substances; it affects the pharmacokinetics of many drugs; it regulates chemical modifications of some ligands; it shows (pseudo-) enzymatic properties; it inactivates some toxic compounds; and it displays antioxidant properties. Importantly, many of these effects result from its unique ability to bind numerous endogenous and exogenous compounds.[7]

The pathogenesis of CDI is strongly related to the harmful effects of toxins (particularly TcdB).[8] Albumin has been shown to have a protective role in some toxin-mediated clinical syndromes. Several animal studies have reported a role of albumin in protecting from endotoxemic shock induced by *E. coli* lipopolysaccharide (LPS). Tokunaga et al[9] conducted a study using a rat model to show that the administration of albumin reduced the myocardial damage in rats with LPS endotoxemia.[9] Similarly, Meziani et al[10] demonstrated the protective effect of human serum albumin (HSA) treatment in experimental endotoxic shock induced in LPS-exposed mice: HSA prevented endothelial dysfuncion and reduced the levels of nitric oxide and superoxide anion release induced by the endotoxin exposure.[10] Thus, we hypothesized that albumin has a direct protective effect on enterocytes exposed to *C. difficile* toxins.

We exposed human epithelial colorectal adenocarcinoma cells (Caco-2) to TcdA or TcdB and analyzed cell metabolic activity in the absence and presence of HSA. Briefly, 1.5×10^4 cells/well were seeded in 96-well plates and exposed for 24 hours to 16 μg/mL of either TcdA or TcdB (Enzo Life Science, Farmingdale, NY) in the absence and presence of 1×10^{-4} M HSA (Sigma-Aldrich, St. Louis, MO). At the end of the exposure, the MTT solution was added (0.5 mg/mL) and incubated for 4 hours at 37°C. Formalized crystals were then dissolved in 4 mM HCl and 0.1% NP40 in isopropanol. The plates were analyzed at 570 nm using a microplate reader (Victor 2, Perkin Elmer, Hopkinton, MA). Untreated control sets were also run under identical conditions.

TcdA exerts no significant effects on Caco-2 metabolic activity both in the absence and in the presence of HSA (Figure 1A). On the contrary, albumin exerts a protective effect on Caco-2 cells from the cytotoxic effect of TcdB (Figure 1B). Figure 1C shows the morphologies of untreated and TcdB-treated Caco-2 cells, in the absence and presence of HSA. Cells treated with TcdB changed into spherical shapes and detached from the surface, and albumin prevented this cytotoxin-associated cellular phenotype.

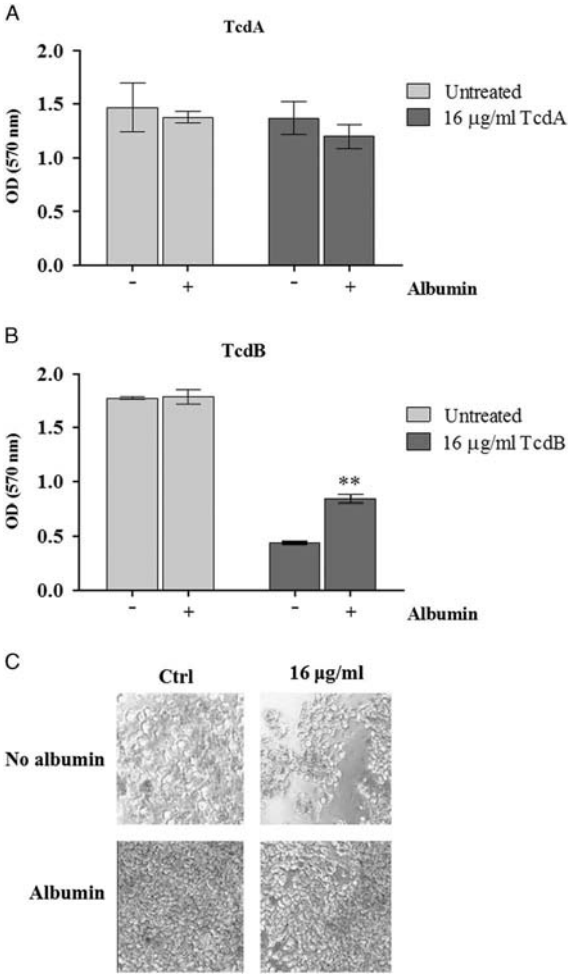

FIGURE 1. Cytotoxic effect of (A) TcdA and (B) TcdB on CaCo-2 cells after treatment with 16 µg/mL for 24 h, in the absence and presence of 1×10^{-4} M human serum albumin, as measured by the MTT cytotoxic assay. Results are shown as the means +/− standard deviations derived from three independent experiments. Statistical significance between means was assessed by Student's *t* test. Statistical significance is considered when *P* values are <.05 (*) and <.01 (**). (C) Morphology of Caco-2 cells, either untreated or exposed to TcdB, in the absence and presence of albumin.

Our experiments demonstrate that the administration of albumin protects enterocytes from *C. difficile* TcdB-induced death (Figure 1). This finding is consistent with a protective role of albumin in CDI rather than being a proxy for underlying disease. A possible explanation is the ability of albumin to scavenge TcdB, which reduces the systemic manifestations of CDI. We believe that our discovery helps elucidate the clinical significance of the known association between albumin levels and the development of CDI.

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