Inflammation of the proximal colon of IL10$^{-/-}$ mice induced by *Helicobacter typhlonius* infection reduces anion secretion and expression of the NaHCO$_3$ cotransporter, NBCe1

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Intestinal inflammation, such as that associated with inflammatory bowel disease (IBD), is reported to cause a marked reduction in intestinal fluid and electrolyte secretion. However, the underlying cause of the reduced secretion is contentious (Seidler *et al*., 2006). In this study, the effects of inflammation of the proximal colon (PC) on electrogenic anion secretion and the expression of associated transporter proteins were investigated. The interleukin 10-knockout (IL10$^{-/-}$) mouse, an established animal model of IBD, was used. In the University of Otago animal facility, IL10$^{-/-}$ mice do not exhibit significant intestinal inflammation. However, when infected with *Helicobacter typhlonius* (IL10$^{-/-}$ infected) the mice develop a severe inflammation of the PC. IL10$^{-/-}$ mice without *H. typhlonius* infection (IL10$^{-/-}$ uninfected) were used as control animals. In addition, wild type mice with and without *H. typhlonius* (WT infected and WT uninfected, respectively) were included to identify possible affects of infection that were independent of inflammation. Electrogenic anion secretion was measured using isolated pieces of PC in the Ussing chamber, and the expression of transport proteins was determined with immunohistochemistry and Western blotting.

The spontaneous short circuit current (I_{sc}) was comparable in the four groups of mice, and the response to forskolin (10 μM mucosal and serosal) was comparable for uninfected and infected WT mice and uninfected IL10$^{-/-}$ mice, whereas the I_{sc} response to forskolin was reduced in the infected IL10$^{-/-}$ mouse ($\Delta I_{sc}^{forskolin}$ IL10$^{-/-}$ controls = 99.36±13.45μA cm^{-2}; $\Delta I_{sc}^{forskolin}$ IL10$^{-/-}$ infected = 60.27±10.95μA cm^{-2}, X±SEM, n = 8), although not significantly. However, comparison of the effects of bumetanide (100 μM serosal), a specific inhibitor of the Na^+-K^+-2Cl^- cotransporter (NKCC1) that drives electrogenic Cl^- secretion, and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 500 μM serosal), an inhibitor of the Na^+-HCO_3^- cotransporter (NBCe1) that drives electrogenic HCO_3^- secretion, indicated that following stimulation with forskolin, in the inflamed tissues there was no change in the bumetanide-sensitive I_{sc}, but a marked reduction in the DIDS-sensitive I_{sc} (ΔI_{sc}^{DIDS} IL10$^{-/-}$ controls = −49±8μA cm^{-2}; ΔI_{sc}^{DIDS} IL10$^{-/-}$ infected = -23±3μA cm^{-2}, X±SEM, n = 8, P<0.05). This suggests possible downregulation of NBCe1 but not NKCC1. Consistent with this the total expression of NKCC1 and its distribution in the colonic epithelium were not altered by inflammation. In contrast, there was a marked reduction in NBCe1 expression in the inflamed colon. In the uninfected IL10$^{-/-}$ mice and both uninfected and infected WT mice, NBCe1 was predominantly expressed in the surface cells, with low levels of expression in the crypts. However, in the inflamed tissues from the infected IL10$^{-/-}$ mice, NBCe1 immunoreactivity was markedly reduced and the total amount of NBCe1 protein was significantly (P< 0.001, n = 6) reduced compared with the control animals. The reduction in NBCe1 expression and associated forskolin-stimulated I_{sc} in the inflamed proximal colon will potentially affect luminal pH regulation and hence growth of luminal bacteria. This may result in dysbiosis, which would contribute to the inflammation. In addition, reduced HCO_3^- secretion will modify the hydration of the secreted mucus, potentially altering the luminal barrier (Muchekehu & Quinton, 2010; Gustafsson *et al*., 2012), which will further exacerbate inflammation.

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