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REFERENCES

1. **Chu CJ**, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002;**122**:1756–62.
2. **Yin J**, Zhang H, Li C, *et al*. Role of hepatitis B virus genotype mixture, subgenotypes C2 and B2 on hepatocellular carcinoma: compared with chronic hepatitis B and asymptomatic carrier state in the same area. *Carcinogenesis* 2008;**29**:1685–91.
3. **Yu MW**, Yeh SH, Chen PJ, *et al*. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005;**97**:265–2.
4. **Yang HI**, Yeh SH, Chen PJ, *et al*. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008;**100**:1134–43.

A distinct subset of submucosal mast cells undergoes hyperplasia following neonatal maternal separation: a role in visceral hypersensitivity?

We recently read with great interest the article published by Barreau *et al* (*Gut* 2008;**57**:582–90) who report, as one of their primary findings, mast cell hyperplasia and increased colonic rat mast cell protease II (RMCP II) activity following neonatal psychological stress. The authors also persuasively demonstrate a potential role for mast

cell-derived nerve growth factor in contributing to increased colonic neural density in this model. In the study we describe here, using a related maternal separation procedure¹ we also observed mast cell hyperplasia. However, this was restricted to the colonic submucosa and characterised by RMCP II immunoreactivity and predominantly blue or blue/red positive cells following Alcian blue/safranin staining, without an associated change in mucosal mast cell (MMC) number.

Maternal separation was carried out using a protocol previously described by our group and known to induce a number of behavioural and gastrointestinal (GI) effects,¹ and adult male animals, at least 11 weeks of age, were used in subsequent studies. To identify mast cells we used sheep anti-RMCP II (1:500; Moredun, Midlothian, UK) and Alcian blue (1% in 0.7 mol/l HCl)/safranin (0.5% in 0.125 mol/l HCl) staining. Analysis of colonic supernatants for RMCP II release was carried out using an enzyme-linked immunosorbent assay (ELISA; Moredun).

Similarly to Barreau *et al* we identified extensive RMCP II positive staining along the crypt mucosa axis (non-separated (NS), 49.5 (SEM 3.7) mast cells/mm², n = 10, maternally separated (MS), 53.1 (SEM 2.5) mast cells/mm², n = 15, p > 0.05) in rat colon; though we did not observe any significant difference between NS and MS animals. We also identified, albeit in much

fewer numbers than those recorded in the mucosa, RMCP II positive cells in the submucosa (fig 1B,C). Moreover, the number of submucosal RMCP II immunoreactive cells was significantly increased following neonatal psychological stress relative to NS controls (fig 1A). Although RMCP II is considered a mucosal mast cell (MMC) marker² it has previously been reported that a small percentage of connective tissue mast cell (CTMC) also stains positively for this chymase in the rat colon.³ Further characterisation of submucosal mast cells revealed that they were insensitive to paraformaldehyde fixation and the majority stained blue or blue/red (fig 1E,F), with few, if any, cells staining red (data not shown) following the Alcian blue/safranin sequence, consistent with a CTMC phenotype.^{2,3} As observed with RMCP II immunostaining, Alcian blue/safranin positive cells were significantly increased in MS animals (fig 1D).

As it has been previously demonstrated by others that the mast cell stabiliser doxantrazole reversed visceral hypersensitivity in rat models of early life stress,⁴ we measured the visceral pain response in our MS animals 30 min following intra-peritoneal administration of doxantrazole (5 mg/kg) or vehicle in order to determine whether increased CTMC may contribute to visceral pain. Colorectal distension was performed by inserting a latex balloon (6 cm) into the colon. The balloon was distended from 0 to

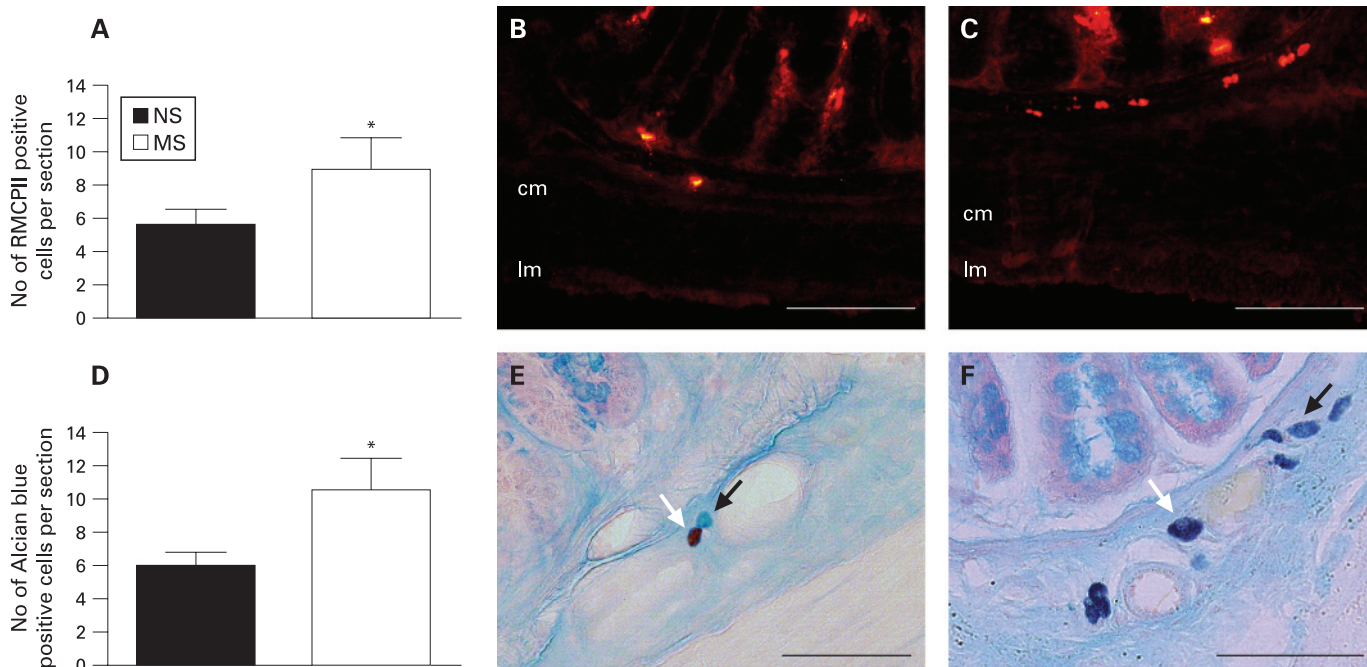


Figure 1 Significantly more rat mast cell protease II (RMCP II) positive cells were localised in the submucosa of maternally separated (MS) animals compared to non-separated (NS) controls (A, B and C; n = 10, p = 0.05, one-tailed Student t test). As observed with RMCP II immunoreactivity, Alcian blue/safranin positive mast cells were significantly increased in MS colon (D; n = 10, p = 0.02, one-tailed Student t test). Alcian blue (E and F, black arrows) and Alcian blue/safranin (E and F, white arrows) positive mast cells were detected in the submucosa of NS and MS animals. The Alcian blue/safranin staining is not homogenous throughout the tissue section, and in some sections represents the total number of cells counted per section. Goblet cell staining can also be seen in the colonic crypts in both E and F. Values are the means; error bars represent the SEM. Scale bars = 100 μ m. cm = circular muscle, lm = longitudinal muscle.

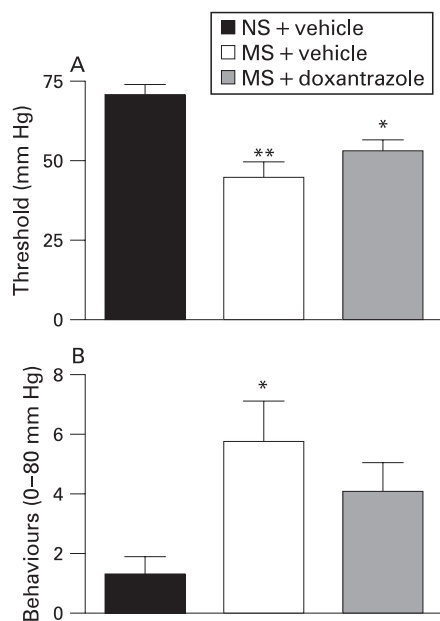


Figure 2 Maternally separated (MS) animals display a decreased threshold, the pressure at which the first pain behaviours were observed, during colorectal distension relative to non-separated (NS) animals. Pre-treatment with the mast cell stabiliser doxantrazole (5 mg/kg, intraperitoneally) did not significantly alter the threshold in MS animals. (A; $n = 7-11$). Furthermore, doxantrazole had no significant effect on cumulative abdominal contractions observed during ramp distension (0–80 mm Hg, 8 min) (B; $n = 7-13$). Values are means; error bars represent the SEM. * $p \leq 0.05$; ** $p \leq 0.01$; one-way analysis of variance followed by Tukey's multiple comparison test.

80 mm Hg over 8 min, during which the threshold pressure (mm Hg) and the cumulative number of visceral pain behaviours were visually recorded.¹ As expected, MS animals (range, 28.8–71.2 mm Hg) displayed pain behaviours at a lower distension pressure (threshold) than NS animals (range, 55.1–79.6 mm Hg, fig 2A, $p \leq 0.01$); however, this was insensitive to mast cell stabilisation ($p \leq 0.05$, MS + doxantrazole vs NS). In addition, the total number of pain behaviours observed over 8 min from 0 to 80 mm Hg was not significantly altered by doxantrazole pre-treatment (fig 2B). In order to determine whether MS animals displayed increased protease release per se, and to confirm the stabilising effect of doxantrazole, full thickness colonic segments (3 cm) were removed from animals immediately post CRD, were stimulated with anti-rat IgE (1:250; MP Biomedical, Solon, Ohio, USA) for 30 min in Hanks' balanced salt solution (Sigma, Poole, UK) bubbled with carbogen (95% oxygen/5% carbon dioxide) at 37°C in a shaking water bath and supernatants were analysed by ELISA. MS animals released approximately 50% more RMCPII following

anti-IgE stimulation relative to NS animals (NS, 0.07 (SEM 0.02) ng/mg, $n = 7$; MS, 0.14 (SEM 0.04) ng/mg, $n = 9$). MS tissues taken from animals treated with doxantrazole displayed similar RMCPII release to NS animals (MS + doxantrazole, 0.07 (SEM 0.01) ng/mg, $n = 10$). These data suggest that MS tissues, consistent with hyperplasia of RMCPII containing mast cells, display increased protease release, although the exact source, MMC or CTMC, is unclear.

Nonetheless, our data suggest that the role of mast cells in the manifestation of early-life stress-induced hypersensitivity is not sensitive to acute mast cell stabilisation in adulthood. This premise is bolstered by the findings by Barreau *et al* describing the adolescent period as being critical for GI-associated neuro-immune alterations. Whether this period is also critical in CTMC development or migration or in their elaboration of protease content remains to be determined.

While subtle but significant differences between our maternal separation protocol and that of Barreau *et al* (eg, litter vs single pup separation and rat strain) may account for the discrepancy in MMC hyperplasia between our studies, our data, in concert with that of Barreau *et al*, further support a link between detrimental early life events and altered mast cell development or function in adulthood. Moreover, our data emphasise the heterogeneity that exists in the GI tract with respect to mast cell populations, and implicates a novel role for all, or a subset of CTMC (RMCPII containing) in contributing to the development of functional GI disorders as a result of early life stress.

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Competing interests: None declared.

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REFERENCES

1. O'Mahony SM, Marchesi JR, Scully P, *et al*. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biol Psychiatry* 2009;65:263–7.
2. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997;77:1033–79.
3. Gibson S, Miller HR. Mast cell subsets in the rat distinguished immunohistochemically by their content of serine proteinases. *Immunology* 1986;58:101–4.
4. Barreau F, Cartier C, Ferrier L, *et al*. Nerve growth factor mediates alterations of colonic sensitivity and mucosal barrier induced by neonatal stress in rats. *Gastroenterology* 2004;127:524–34.

Authors' reply

In their letter, Hyland *et al* report differences between data they obtained on mast cell phenotype and the role in visceral hypersensitivity to colorectal distension in adult rats previously submitted to maternal separation, and those we obtained using the same experimental protocol.¹ They confirm the existence of colonic mast cell hyperplasia in maternally deprived rats, but they found it localised in the submucosa, while in our paper it was clearly localised in the mucosa. It has been shown that protease phenotype of mast cells and their locations in epithelium, lamina propria and submucosa may vary according to the different phases of an acute inflammation.² Since maternal deprivation is known to induce a moderate colonic inflammation in adults, we can speculate that the inflammatory state was different in our rats and those of Hyland *et al*, because of differences of strains or differences in rearing conditions.

According to the characteristics of mucosal mast cells (MMCs) and connective tissue mast cells (CTMCs) provided by Metcalfe *et al*,³ mast cells described in our paper are clearly MMCs on the basis of size and content of rat mast cell protease II (RMCPII). Hyland *et al* propose that the mast cells they described are CTMCs, since it has been suggested in one paper that some intestinal CTMCs may be RMCPII positive. We did not find any clear evidence of this statement in the paper they cite, and we agree with the conclusion that the "exact source, MMC or CTMC, is unclear".

Our paper indicates that the amount of RMCPII released by colonic tissue in basal conditions, or under stimulation by substance P or a calcium ionophore, is increased in maternally deprived rats. Interestingly, Hyland *et al* confirm this increased release using another stimulating agent. We have also shown in another paper⁴ that the hypersensitivity to colorectal distension, characterising maternally deprived rats, was abolished by the mast cell stabiliser doxantrazole. Hyland *et al* do not confirm this result. They verified that doxantrazole treatment was effective, since it reduced the RMCPII release after anti-IgE stimulation. In our study, we used a different protocol