The Presence in Blood of Both Glycosaminoglycan and Mucosal Mast Cell Protease following Systemic Anaphylaxis in the Rat

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Abstract

The appearance in blood of rat mast cell protease II (RMCP II) and glycosaminoglycan (GAG) was examined in normal and *Nippostrongylus brasiliensis*-primed rats challenged intravenously with worm antigen. Systemic release of these two products occurred only in immune recipients of antigen; substantial levels of RMCP II were also present in the intestinal perfusates of these same rats and there was depletion of both RMCP II and mucosal mast cells (MMC) from the intestinal mucosa. Depletion of MMC was evident after staining for proteoglycan or for serine esterase and the mast cell counts with both histochemical techniques were highly correlated. Taken together, the results suggest that MMC are likely to be the principal source of secreted GAG and RMCP II.

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During the immune expulsion of the gastrointestinal nematode *Nippostrongylus brasiliensis* from the rat, there is discharge of granule contents of mucosal mast cells (MMC), and depletion of proteoglycan from these cells [1]. Following intravenous challenge of primed rats with *N. brasiliensis* worm antigen, extensive mucosal damage ensues which is accompanied by the systemic and enteric release of a specific neutral proteinase: rat mast cell protease II (RMCP II) [2] derived from MMC, and depletion of MMC from the jejunal mucosa [3]. The present study was undertaken to analyse body fluids for the presence of MMC-derived proteoglycan during systemic anaphylaxis in the rat.

15 female outbred Wistar rats, immunised 3 and 8 weeks earlier by subcutaneous injection with 6,000 *N. brasiliensis* larvae [4], were allocated randomly to 3 groups of 4 rats and 1 group of 3 rats and were challenged intravenously with 1,000, 500 or 250 worm equivalents (w.e.) of adult whole worm antigen or saline. Worm antigen was prepared as described previously [5]. A further group of 4 naive rats was challenged with 500 w.e. intravenously. 1 h after intravenous challenge, animals were anaesthetised with ether and bled out. Blood was collected into the anticoagulant 'Thrombotect' (Abbott) and the plasma was separated by centrifugation and stored at -70 °C. Serum,

small intestinal perfusates and samples of jejunum were collected and the concentrations of RMCP II in these samples were measured by radial immunodiffusion [2, 3]. Additionally, adjacent segments of jejunum were placed in either Carnoy's fluid or 4% para-formaldehyde prepared with phosphate-buffered saline. Tissues were embedded in wax and sections were stained with either Toluidine blue [6] or with naphthol AS-D chloroacetate to demonstrate serine

esterases [7]. Plasma, gut perfusates and gut homogenates were analysed for the presence of glycosaminoglycan (GAG) by a modification of the competitive heparin binding assay previously described [8], in which poly-brene-Sepharose was substituted for protamine-Sepharose as the binding reagent and the radioactive tracer and standard were heparan sulphate rather than heparin.

RMCPH was present only in the sera of immune rats challenged with worm antigen (fig. 1) and in these same rats there was a 10-fold increase in the concentration of GAG in plasma when compared with immune recipients of saline or naive rats given antigen (fig. 1). Although the release of RMCPH and GAG into the blood was, in this experiment, independent of the dose of antigen, there was a highly significant correlation between the concentrations of RMCPH and GAG ($y = 2,110 + 17 x$, $r = 0.93$, $p < 0.001$; fig. 2),

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3000

1000

250 500 750 Dose (w.e.)

40

20

1000

200 400 600 800 μg RMCPH/ml serum

Fig. 2. Regression analysis of the concentrations of RMCPH in serum against GAG in plasma ($y = 2,110 + 17 x$, $r = 0.93$, $p < 0.001$). Plasma was not obtained from one rat given 500 w.e.

30

20

10

0

Fig. 1. a Total recoveries of RMCPH (mean $\pm$ SEM) in intestinal perfusates ($\bullet$) and concentration of enzyme in jejunal mucosa ($\mu\text{g/g}$ wet weight tissue, $\blacksquare$) of immune rats given worm antigen or saline (dose = 0). The number of mucosal mast cells (MMC) per vil-lus crypt unit (VCU) detected with Toluidine blue (mean $\pm$ SEM, $\circ$) are also shown. Individual symbols showing the concentrations of RMCPH in perfusates ($\blacklozenge$) and gut homogenates ($\square$) and the number of MMC ($\circ$) in naive rats challenged with 500 w.e. of antigen are also plotted, b The concentrations of RMCPH in serum (mean $\pm$ SEM $\bullet$) and glycosaminoglycan (GAG) in plasma (mean $\pm$ SEM $\blacksquare$) are plotted against the dose of worm antigen. Values from immune rats injected with saline are plotted at the zero dose and in naive rats given antigen the values are plotted at 500 w.e. for RMCPH ($\circ$) and for GAG ($\square$).

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10

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MMC/VCU (Esterase)

Fig. 3. Regression analysis demonstrating a highly significant correlation between the numbers of MMC enumerated after staining with Toluidine blue and with naphthol AS-D chloroacetate ($y = -2.3 + 1.1 x$, $r = 0.98$, $p < 0.001$).

which would suggest that they may have been released from the same source.

Intestinal perfusates from primed, challenged rats contained 1,144–2,085 μg RMCPII (fig. 1), whereas perfusates from controls were devoid of this enzyme (fig. 1). There was a concomitant, highly significant ($p < 0.001$) depletion of RMCPII and MMC from the mucosa of primed, challenged rats when compared with control values (fig. 1). Analysis of gut perfusates and homogenates showed GAG to be present within each sample. No decrease from jejunum or increase within the perfusate was detected. The analysis of these tissues is, however, likely to be complicated by endogenous GAG other than that contained in the mast cells.

Mast cell depletion from the mucosa was detected both with Toluidine blue (pH 0.5) and with naphthol AS-D chloroacetate; the correlation between the numbers of mast cells detected with these two methods was highly significant ($y = -2.3 + 1.1 x$, $r = 0.98$, $p < 0.001$;

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fig. 3), suggesting that proteoglycan and serine ester-ases were depleted concomitantly from discharging MMC.

The results of this study are consistent with data from previous experiments [2] except that shock was severe with the lowest dose of worm antigen and was not augmented by further increasing the challenge dose. There was, as previously described [2], substantial secretion of RMCPII into the gut lumen and a concomitant fall in the mucosal concentration of this enzyme. These changes were paralleled by a reduction in the number of MMC detected with either Toluidine blue or naphthol AS-D chloroacetate. Whilst we were unable to detect changes in mucosal levels of GAG, probably for technical reasons associated with a high background of tissue GAG, there was a tenfold increase in the concentration of GAG in plasma which was highly correlated with the release, into serum, of RMCPII. Although the distribution of RMCPII in tissues other than the gut have not yet been published, our own studies have shown that, apart from the lung in which RMCPII is increased after infection with *N. brasiliensis* and which, at most, contains 300 μg RMCPII/g wet weight, most other organs contain little or none of this enzyme [9]. Clearly, therefore, the major source of secreted RMCPII is the enteric mucosa, and it would seem likely that GAG present in plasma is also released from this site with MMC being the most obvious source of these two products.

More conclusive evidence of the mucosal mast cell as the source of secreted GAG must await further analysis. However, it is relevant to note that MMC granule proteoglycan is, like that from the murine mast cell cultured from bone marrow [10], non-heparin proteoglycan [11], and that the latter cell releases chondroitin sulphate E in response to immunological stimuli [12]. By contrast the heparin present in rat peritoneal mast cells remains within the granule matrix, apparently complexed with the insoluble chymase (RMCPI) [13]. If, as the present study suggests, granule products of MMC are highly soluble, this is yet another example of the biochemical and functional differences between MMC and connective tissue mast cells.

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References

- Miller, H.R.P.; Walshaw, R.: Immune reactions in mucous membranes. IV. Histochemistry of intestinal mast cells during helminth expulsion in the rat. *Am. J. Path.* 69: 195–208 (1972).
- King, S.J.; Miller, H.R.P.: Anaphylactic release of mucosal mast cell protease and its relationship to gut permeability in *Nippostrongylus*-primed rats. *Immunology* 51: 653–660 (1984).
- Miller, H.R.P.; Woodbury, R.G.; Huntley, J.F.; Newlands, G.: Systemic release of mucosal mast cell protease in primed rats challenged with *Nippostrongylus brasiliensis*. *Immunology* 49: 471–479 (1983).
- Nawa, Y.; Miller, H.R.P.: Protection against *Nippostrongylus brasiliensis* by adoptive immunization with immune thoracic duct lymphocytes. *Cell. Immunol.* 37: 51–60 (1978).
- Nawa, Y.; Miller, H.R.P.; Hall, E.; Jarrett, E.E.E.: Adoptive transfer of total and parasite-specific IgE responses in rats infected with *Nippostrongylus brasiliensis*. *Immunology* 44: 119–123 (1981).
- Enerback, L.: Mast cells in rat gastrointestinal mucosa. II. Dye binding and metachromatic properties. *Acta path, microbiol. scand.* 66: 303–312 (1966).
- Newlands, G.F.J.; Huntley, J.F.; Miller, H.R.P.: Concomitant detection of mucosal mast cells and eosinophils in the intestines of normal and *Nippostrongylus*-immune rats. A re-evaluation of histochemical and immunocytochemical techniques. *Histochemistry* (in press).
- Dawes, J.; Pepper, D.S.: A sensitive competitive binding assay for exogenous and endogenous heparins. *Thromb. Res.* 27: 387–396 (1982).
- King, S.J.; Miller, H.R.P.; Woodbury, R.G.; Newlands, G.F.J.: Gut mucosal mast cells in *Nippostrongylus*-primed rats are the major source of secreted rat mast cell protease II following systemic anaphylaxis. (submitted for publication, 1984).
- Razin, E.; Stevens, R.L.; Akiyama, F.; Schmid, K.; Austen, K.F.: Culture from mouse bone marrow of a subclass of mast cells possessing a distinct chondroitin sulfate proteoglycan with glycosaminoglycans rich in N-acetylgalactosamine-4,6-disulfate. *J. biol. Chem.* 257: 7229–7236 (1982).
- Jarrett, E.E.E.; Haig, D.M.: Mucosal mast cells in vivo and in vitro. *Immunol. Today* 5: 115–117 (1984).
- Razin, E.; Mencia-Huerta, J.M.; Stevens, R.L.; Lewis, R.A.; Liu, F.T.; Corey, E.J.; Austen, K.F.: IgE-mediated release of leukotriene C₄, chondroitin sulfate E proteoglycan, β -hexosaminidase and histamine from cultured bone marrow derived mast cells. *J. exp. Med.* 757: 189–201 (1983).
- Schwartz, L.B.; Riedel, C.; Caulfield, J.P.; Wasserman, S.I.; Austen, K.F.: Cell association of complexes of chymase, heparin proteoglycan, and protein after degranulation by rat mast cells. *J. Immun.* 126: 2071–2078 (1981).