Depletion of mucosal mast cell protease by corticosteroids: Effect on intestinal anaphylaxis in the rat
(helmint infection/ atypical mast cell/ serine protease/ glucocorticoids/ immediate hypersensitivity)

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ABSTRACT Rats primed by infection with the intestinal nematode Nippostrongylus brasiliensis and challenged intravenously with soluble whole-worm antigen undergo systemic anaphylactic shock. The primary lesions are in the gut and include increased permeability of the mucosa together with release, into enteric secretions, of a mucosal mast cell (MMC)-specific serine proteinase, rat mast cell protease II (RMCP-II). This enzyme is also released into the blood of shocked rats. These manifestations of anaphylaxis were abolished in rats previously treated with corticosteroids (methylprednisolone acetate, 25 mg per kg of body weight, 48 and 24 hr before i.v. challenge with antigen). Suppression of the response was associated with depletion of RMCP-II and of MMC from the intestinal mucosa. Depletion occurred 4-24 hr after treatment with as little as 1 mg of methylprednisolone per kg. By contrast, neither connective tissue mast cells nor serum levels of parasite-specific IgE were depleted in rats given 2 x 25 mg of methylprednisolone per kg. The capacity of unprimed treated rats to mount passive cutaneous anaphylaxis was, however, impaired.

Corticosteroids are potent, widely used anti-inflammatory drugs, and their therapeutic value in the treatment of allergic conditions in man and domestic animals is well known. Although these drugs act at a number of different levels, perhaps their most significant anti-allergic effect is in preventing the generation and release of inflammatory mediators (1). For example, they suppress histamine release from isolated rat and murine mast cells (2, 3), and they act indirectly to prevent the generation of secondarily formed mediators (4-6).

Systemic anaphylaxis in the rat is abrogated by prior treatment of sensitized animals with corticosteroids (7). The major shock organ responding to anaphylaxis in this species is the small intestine (8), and the lesions associated with intestinal anaphylaxis include hyperaemia, secretion of mucus, and epithelial shedding (9-11). The development of intestinal lesions is associated with the release, into the blood circulation and into the gut lumen, of a mucosal mast cell (MMC)-derived neutral proteinase, rat mast cell protease II (RMCP-II) (10, 11), and recent studies have implicated this enzyme in the generation of intestinal epithelial permeability (11).

Because RMCP-II is a highly soluble product of MMC (12) and is antigenically distinct from insoluble chymotrypsin-like enzyme (RMCP-I) in connective tissue mast cells (13), its release into the blood circulation or gut lumen provides a unique and highly specific marker of in vivo activation of MMC. We have, therefore, measured the concentration of this enzyme within the tissues, in the intestinal secretions, and in blood in order to analyze the anti-anaphylactic activity of corticosteroids and to measure their effect on the mucosal mast cell subpopulation in rats subjected to anaphylactic shock.

MATERIALS AND METHODS

Animals. Male and female outbred Wistar rats matched for age (30-36 weeks) and weight (350-450 g) were used. They were watered and fed ad lib, but food was withdrawn 24 hr before challenge.

Parasitological Techniques. The methods used to culture the nematode parasite Nippostrongylus brasiliensis and to infect rats have been described (14). Adult whole-worm antigen was prepared as described (15).

Treatment of Experimental Animals. Rats were allocated to groups and were treated with saline or methylprednisolone acetate (25 mg per kg of body weight) by intramuscular injection 24 and 48 hr before they were challenged intravenously with Evan’s blue (2.5 mg per 100 g of body weight). The latter was dissolved either in saline alone or in saline containing 500 worm equivalents (w.e.) (15) of worm antigen. Treatment schedules are summarized in Table 1.

Quantification of Evan’s Blue and RMCP-II. One hour after intravenous challenge, rats were exsanguinated under ether anesthesia, and their intestines were excised. Each intestine was perfused with saline and the concentration of Evan’s blue in the perfusate was determined spectrophotometrically (11). The following tissues were collected: jejunum (25 cm distal to the pylorus); ileum (10 cm proximal to the ileo caecal valve); mesenteric lymph node (MLN) (divided into two portions, one for histology); and the right lobe of the lung. Each specimen was weighed and homogenized in 3 vol of 0.15 M KCl (16). The concentrations of RMCP-II in the tissue homogenates, gut perfusates, and serum were measured by radial immunodiffusion (11).

Histology and Cell Counts. (i) Carnoy fixation. The following tissues were placed in Carnoy’s fluid; jejunum and ileum (specimens adjacent to those used for protease determination), MLN, and the left lobe of the lung. The tissues were processed and stained with Alcian blue/Safranin as described (16).

(ii) Paraformaldehyde (4%) in phosphate-buffered saline. Portions of jejunum and ileum and ear pinnae (from naïve rats) were fixed for 6 hr, transferred to 70% ethanol, and stored at 4°C overnight. The tissues were dehydrated and processed into wax, and sections were stained for nonspecific esterase with the substrate naphthol AS-D chloroacetate (Fig. 1) (17). Adjacent sections were stained with Alcian blue to show mast cells (16).

Mast cells in both jejunum and ileum were counted per villus crypt unit (VUC) as described (16). Mast cells were

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Abbreviations: MMC, mucosal mast cell; CTMC, connective tissue mast cell; RMCP, rat mast cell protease; w.e., worm equivalent; MLN, mesenteric lymph node; VCU, villus crypt unit; PCA, passive cutaneous anaphylaxis.
not enumerated in lung and MLN, but mast cell density was scored on an arbitrary scale.

Sections of ear pinnae were examined microscopically and the total number of mast cells was enumerated. The area of tissue in which mast cells were counted, excluding cartilage, was measured by projecting the sections onto equiweight paper using a photoenlarger and then calculating the area by weight analysis of the tissue outline.

Passive Cutaneous Anaphylaxis (PCA). Rats were bled via the tail vein before saline or drug treatment and again immediately before challenge with worm antigen. Sera were harvested and double dilutions were injected intradermally into the shaved backs of recipient rats, which were challenged intravenously 48 hr later with worm antigen (1000 w.e.) and Evan’s blue (18). PCA titers were expressed as the reciprocal of the highest dilution that induced blueing to a diameter of >5 mm on the underside of the skin.

The Effect of Corticosteroid Treatment of PCA. Recipient rats were injected intradermally with double dilutions of a serum sample taken from an animal immunized by two previous infections with *N. brasiliensis*. The recipients were immediately injected intramuscularly with either saline or methylprednisolone (25 mg per kg of body weight). These treatments were repeated 24 hr later, and PCA titers were determined after a further 24 hr.

RMCP-II Release as a Consequence of Corticosteroid Treatment. Fifteen naive rats, allocated to three groups, were bled via the tail vein before receiving intramuscular injections of saline, methylprednisolone acetate (25 mg per kg of body weight), or betamethasone (2 mg per kg of body weight) and were bled, 1, 4, and 24 hr later; rats given betamethasone were exsanguinated at 24 hr, and the remainder were given a second dose of saline or methylprednisolone. Blood samples were taken 1 and 4 hr later, and the remaining rats were exsanguinated 24 hr after the second injection. Samples of jejunum were collected for measurement of RMCP-II concentrations and of the numbers of MMC. Sera were analyzed for RMCP-II using an ELISA technique as described (10).

Statistical Treatment of Results. Data were analyzed using a Tektronix microcomputer using either a Student’s two-tailed *t* test or, where necessary, a Mann–Whitney *U* test. Simple regression analysis was performed using the same computer facilities.

RESULTS

Intestinal Permeability and Secretion of RMCP-II. A highly significant (*P* < 0.001, Student’s *t* test) 4-fold increase in the concentration of Evan’s blue occurred in the intestinal lumen of sensitized animals (group I) challenged intravenously with worm antigen (Table 1). This response was totally suppressed in sensitized rats that had been previously treated with methylprednisolone (group II in Table 1).

Similarly, RMCP-II was released into the blood and into the intestinal lumen of rats in group I, but this response was again totally suppressed (*P* < 0.001, Mann–Whitney *U* test) in rats previously treated with methylprednisolone (group II, in Table 1). Saline injection of either untreated or drug-tREATED sensitized rats and antigen injection into either untreated or drug-treated naive rats induced no significant alteration of mucosal permeability or release of RMCP-II (Table 1).

### Table 1. Measurement of gut permeability and RMCP-II release during anaphylaxis in normal and corticosteroid-treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th></th>
<th>Pretreatment/challenge</th>
<th>Evan’s blue, µg per perfusate</th>
<th>Systemic RMCP-II, µg per ml of serum</th>
<th>Enteric RMCP-II, µg per perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Immune</td>
<td>6</td>
<td>S/Ag</td>
<td>406 ± 41*</td>
<td>90 ± 19*</td>
<td>797 ± 250*</td>
</tr>
<tr>
<td>II</td>
<td>Immune</td>
<td>6</td>
<td>C/Ag</td>
<td>88 ± 8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Immune</td>
<td>6</td>
<td>S/S</td>
<td>104 ± 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>Immune</td>
<td>6</td>
<td>C/S</td>
<td>124 ± 8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>Naive</td>
<td>5</td>
<td>S/Ag</td>
<td>64 ± 4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>Naive</td>
<td>5</td>
<td>C/Ag</td>
<td>51 ± 6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>Naive</td>
<td>5</td>
<td>S/S</td>
<td>72 ± 5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VIII</td>
<td>Naive</td>
<td>5</td>
<td>C/S</td>
<td>69 ± 5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Immune animals were infected 1 and 3 weeks previously with 5000 *N. brasiliensis* L1, S, saline; C, methylprednisolone acetate; Ag, 500 w.e. of whole worm antigen. Values represent mean ± SEM.

*P* < 0.001 when compared with each group (Student’s *t* test).

*P* < 0.001 when compared with each group (Mann–Whitney *U* test).
Table 2. Effect of anaphylaxis and corticosteroid treatment on distribution of RMCP-II in jejunum, ileum, lung, and MLN in immune and naive rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Jejunum μg/gram</th>
<th>Ileum μg/gram</th>
<th>Lung μg/gram</th>
<th>MLN μg/gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1780 ± 120</td>
<td>471 ± 19</td>
<td>219 ± 24</td>
<td>86 ± 16</td>
</tr>
<tr>
<td>II</td>
<td>215 ± 25*</td>
<td>106 ± 10*</td>
<td>126 ± 16</td>
<td>&lt;10²</td>
</tr>
<tr>
<td>III</td>
<td>1840 ± 84</td>
<td>517 ± 13</td>
<td>182 ± 17</td>
<td>90 ± 0</td>
</tr>
<tr>
<td>IV</td>
<td>165 ± 33*</td>
<td>98 ± 5*</td>
<td>117 ± 14</td>
<td>&lt;10³</td>
</tr>
<tr>
<td>V</td>
<td>534 ± 51</td>
<td>195 ± 10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>VI</td>
<td>252 ± 57*</td>
<td>61 ± 5*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>576 ± 51</td>
<td>200 ± 6</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>VIII</td>
<td>210 ± 30*</td>
<td>66 ± 0*</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Groups and treatments are as described in Table 1. Values represent mean ± SEM.

*P < 0.001. Analysis of effect of corticosteroid treatment (Student’s t test).
†P < 0.01.
‡P < 0.001 (Mann–Whitney U test).
§P < 0.05.

effect on lung mast cells in naive rats but slightly decreased the numbers of mast cells in the cortex of MLN (Table 3).

The Response of Connective Tissue Mast Cells to Corticosteroid Treatment. Corticosteroid treatment did not alter the numbers of mast cells in the ear pinnae of naive rats. Recipients of saline had 56 ± 4 mast cells per mm² in corticosteroid-treated rats, there were 53 ± 3 mast cells per mm² of tissue. Similarly, antigen challenge of naive rats was without effect on connective tissue mast cells (CTMC) (55 ± 6 mast cells per mm²). The numbers of CTMC stained with Alcian blue when compared with the numbers containing esterase activity were, by simple regression analysis, highly significantly correlated (y = 12.8 + 0.76x; r = 0.81; P < 0.001).

PCA Reactivity of Serum After Corticosteroid Treatment. Serum taken from rats before they were treated with methylprednisolone (PCA titer, 280 ± 114) and serum taken from the same rats immediately before antigen challenge (PCA, 280 ± 114) had identical PCA values. Similarly, PCA titers measured before saline treatment of immune control rats (176 ± 39) were unaltered 48 hr later.

Effect of Corticosteroid Treatment on PCA. The PCA titer (40 ± 0) in naive rats injected intradermally with immune serum and then treated with methylprednisolone was significantly (P < 0.001) decreased when compared with similarly sensitized rats (352 ± 78) treated with saline.

Serum Levels of RMCP-II After Corticosteroid Treatment. The concentration of RMCP-II in sera obtained 1, 4, 24, and 48 hr after treatment with methylprednisolone and 1, 4, and 24 hr after treatment with betamethasone was determined by ELISA. At no time was RMCP-II detected in the blood. There was, however, depletion of MMC after treatment with either methylprednisolone (3.7 ± 0.5 MMC per VCU) or

Table 3. Quantification of MMC in jejunum, ileum, lung, and MLN in normal and corticosteroid-treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Lung</th>
<th>MLN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alcian blue</td>
<td>Esterase</td>
<td>Alcian blue</td>
<td>Esterase</td>
</tr>
<tr>
<td>I</td>
<td>29.6 ± 1.7</td>
<td>30.0 ± 2.0</td>
<td>15.9 ± 1.5</td>
<td>16.0 ± 1.4</td>
</tr>
<tr>
<td>II</td>
<td>3.3 ± 0.3*</td>
<td>3.6 ± 0.2*</td>
<td>2.2 ± 0.4*</td>
<td>2.4 ± 0.4*</td>
</tr>
<tr>
<td>III</td>
<td>32.6 ± 1.0</td>
<td>32.9 ± 1.5</td>
<td>17.4 ± 0.8</td>
<td>18.0 ± 1.0</td>
</tr>
<tr>
<td>IV</td>
<td>3.8 ± 0.4*</td>
<td>4.0 ± 0.3*</td>
<td>2.8 ± 0.3*</td>
<td>2.8 ± 0.1*</td>
</tr>
<tr>
<td>V</td>
<td>6.8 ± 0.5</td>
<td>7.7 ± 0.3</td>
<td>4.0 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>VI</td>
<td>2.1 ± 0.2*</td>
<td>2.5 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>1.3 ± 0.2*</td>
</tr>
<tr>
<td>VII</td>
<td>7.5 ± 0.5</td>
<td>8.3 ± 0.5</td>
<td>4.2 ± 0.2</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>VIII</td>
<td>2.4 ± 0.2*</td>
<td>2.4 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>1.4 ± 0.1*</td>
</tr>
</tbody>
</table>

Groups and treatments are as described in Table 1. Values represent mean ± SEM. Values for MMC/VCU when stained with esterase showed a highly significant correlation with those stained with Alcian blue: for jejunum (y = 0.45 + 0.99x; r = 0.99) and for ileum (y = 0.07 + 1.0x; r = 0.99).

Subjective mast cell density: (+ + +) substantial increase when compared with naive controls (+).

*P < 0.001 significant depletion of mucosal mast cells after corticosteroid treatment.

Some depletion of mast cells after corticosteroid treatment.
betamethasone (5.6 ± 0.2 MMC per VCU) when compared with mast cell numbers in control rats (10.3 ± 0.5 MMC per VCU) treated with saline. The numbers of MMC detected by esterase stain and by Alcian blue were similar, and by regression analysis there was a highly significant correlation between the two staining methods (y = 0.19 ± 0.94x; r = 0.93; P < 0.001). Similarly, the concentration of RMCP-II was decreased (P < 0.001) in the jejunum of rats treated with either methylprednisolone (315 ± 48 μg/g) or betamethasone (493 ± 33 μg/g) when compared with saline controls (784 ± 24 μg/g). There was, in addition, a highly significant correlation between the numbers of MMC detected with esterase stain and the concentration of RMCP-II in the jejunum of the three groups of rats (y = 164 + 61x; r = 0.87; P < 0.001).

Dose–Response Relationship. Rats immunized by two previous infections with N. brasiliensis were injected intramuscularly with saline or with 1, 5, or 25 mg of methylprednisolone acetate per kg of body weight 24 and 48 hr before they were killed and bled out. When compared with saline-treated controls, methylprednisolone caused a dose-dependent depletion of RMCP-II within 24 hr from the mucosa (Table 4). The data fitted the following regression equation: y = x/−0.002 + 0.004x (r = 0.81) and indicated that 5 mg of methylprednisolone per kg of body weight was as effective in depleting MMC from the jejunum as was 25 mg/kg. The decrease in the numbers of jejunal MMC (esterase) was highly correlated with the loss of jejunal RMCP-II (r = 0.98).

Time Course of Mast Cell Changes. Immune rats were allocated to groups and given intramuscular injections of 25 mg of methylprednisolone or saline per kg of body weight. Groups of four rats were killed 1, 4, and 24 hr after prednisolone treatment and four rats were killed 24 hr after saline treatment. Samples of jejunum were collected for histology and for measurement of RMCP-II concentrations. No significant depletion of either mast cells or RMCP-II was detected until 24 hr after injection of corticosteroids (Table 4). These data indicate that mast cell changes occur between 4 and 24 hr after administration of steroid.

**DISCUSSION**

The neutral serine proteinase RMCP-II is located uniquely within the granules of a subset of mast cells commonly referred to as "atypical" or "mucosal mast cells" (19), and the two principal effects of corticosteroid treatment described in the present study were (i) suppression of the anaphylactic release of this enzyme into intestinal secretions and into blood and (ii) depletion of RMCP-II from the intestinal mucosa. These events may, therefore, be related to the suppression of intestinal anaphylaxis and to our failure to detect systemic or enteric release of RMCP-II in primed antigen-challenged rats.

Our results have confirmed previous reports that corticosteroids suppress anaphylactic pathology in the gut (9), there being no detectable permeability changes, and no detectable immune rats challenged with worm antigen (Table 1). In addition, they extend earlier observations on the ability of corticosteroids to suppress the development of intestinal mucosal mastocytosis during nematode infection (20, 21) by showing that fully mature MMC in normal and immune rats are depleted by corticosteroid treatment. By contrast, CTMC in the pinna of the ear were apparently unaffected. This latter finding indicates a further important functional difference between CTMC and MMC in the gut.

Significant depletion of MMC and RMCP-II occurred between 4 and 24 hr after exposure to steroid, and this may explain why in previous studies (7) optimal inhibition of anaphylactic bronchoconstriction was not observed until 12-24 hr after dexamethasone administration. The extreme sensitivity of MMC to steroids was indicated by the fact that treatment with as little as 1 mg of methylprednisolone per kg of body weight caused a significant decrease in their numbers (Table 4).

Depletion of MMC was in every instance paralleled by a loss of RMCP-II from the jejunal mucosa. Both MMC and CTMC were in these studies identified histologically by their content of glycosaminoglycan and of serine esterases. Neither of these granule constituents was depleted from CTMC after steroid treatment, whereas both were lost from MMC. Although depletion of MMC occurred between 4 and 24 hr after treatment, there was no detectable release of RMCP-II into the serum at 1, 4, or 24 hr. The ultimate fate of MMC was not established and must await further study.

Corticosteroid treatment of rats passively sensitized intradermally with immune serum caused a highly significant but incomplete inhibition of passive cutaneous anaphylaxis. The binding of IgE to the mast cell surface is not impaired in vitro by steroids (3, 22) and, in the present study, corticosteroids failed to suppress the level of circulating IgE. Consequently, two mechanisms of inhibition changes in anaphylaxis might operate: (i) inhibition of mediator release from mast cells by corticosteroids and (ii) modification of the response of target organs to these mast cell-derived mediators. The extent to which the intestinal response was suppressed by the known capacity of steroids to decrease permeability in capillary beds (23) or cause vasoconstriction (24) cannot be determined from the present data. It is possible that both mechanisms operate in the suppression of intestinal permeability changes.

Elevation of cAMP (3, 22) and effects on arachidonic acid metabolism (25) and its inhibition of release from the cell membrane by lipomulbin-like proteins (26-28) have been put forward to explain corticosteroid inhibition of mediator release from CTMC. However, it is not clear whether these mechanisms are applicable to MMC, as they differ biochemically and functionally (29) from CTMC. But it is interesting to note that one action of corticosteroids may be to impair mitochondrial function (2), and it is possible that MMC have a greater energy requirement than CTMC.

In summary, pretreatment of sensitized rats with corticosteroids suppresses intestinal anaphylaxis and is associated with a marked depletion of MMC. However, the possibility cannot be ruled out that the depletion of MMC is
an indirect effect resulting from the primary action by corticosteroids on other cell types, such as T lymphocytes. Also, since CTMC remain undepleted from the dermis of the ear pinna, there appears to be a very important difference between the two cell populations in terms of steroid sensitivity, which may be of potential importance in man, where MMC have only recently been identified (30).

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