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Mercuric Chloride-Induced Vasculitis in the Brown Norway Rat: αβ T Cell-Dependent and -Independent Phases

Role of the Mast Cell

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Mercuric chloride induces a necrotizing vasculitis in the Brown Norway (BN) rat. This occurs in two phases, between 1 and 5 days (early) and between 12 and 20 days (late) after initiation of HgCl₂. One outbred and four inbred rat strains were found to be susceptible to early vasculitis, but only the BN strain developed late vasculitis. In the BN strain, treatment with the mAb R73 (anti-αβ TCR) inhibited T cell function, completely prevented the late vasculitis, but had no effect against early vasculitis, indicating that early and late vasculitis is controlled by different genetic and cellular mechanisms. The role of the mast cell in the αβ T cell-independent early phase was studied. Serum concentrations of rat mast cell protease II rose following HgCl₂ treatment, indicating mast cell degranulation. The reagents Doxantrazole and the mAb G63, which suppress mast cell secretory responses, also prevented the rise in rat mast cell protease II and significantly reduced the early vasculitis. The demonstration of an αβ T cell-dependent phase supports previous experimental data that T cells play an important role in the pathogenesis of vasculitis. The presence of an earlier αβ T cell-independent phase is a unique observation. The data support a role for the mast cell in the early vasculitis. The Journal of Immunology, 1997, 159: 5100–5106.

In humans, the systemic vasculitides are a group of diseases characterized by necrotizing leukocytoclastic inflammation in the walls of blood vessels. Numerous classifications have emphasized etiologic and anatomical characteristics, with particular importance placed on the size of the target blood vessel (1). A large number of agents have been implicated in pathogenesis, including infections, malignancy, drugs, and coexisting autoimmune disease (2). Irrespective of the underlying pathogenesis, the involved blood vessel wall becomes infiltrated with a mixture of neutrophils and mononuclear cells. However, the pathogenic role of the individual cell types and the role (if any) of the anti-neutrophil cytoplasm Abs found in association with small vessel vasculitis (3) are less clearly understood.

Mercuric chloride induces an autoimmune syndrome in susceptible strains of rat (4). In the Brown Norway (BN) rat, this includes leukocytoclastic vasculitis which predominantly affects the intestine (5). Although this tissue distribution does not reflect human disease, the histologic features, the association with anti-neutrophil cytoplasm Ab of the anti-myceloperoxidase (MPO) type (6) and the sensitivity to broad spectrum antimicrobial agents (5) are analogous to human vasculitis. In the HgCl₂ model vasculitis is unusual in that it develops in two stages, the first (early vasculitis) occurring within 1 to 5 days of the start of HgCl₂ treatment (7, 8) and the second (late vasculitis), of greater severity, between 12 and 20 days at the same time as the other autoimmune manifestations of the syndrome (5). Early vasculitis is the only tissue manifestation occurring within the first few days of HgCl₂ treatment. The histologic appearances are identical with those of the late vasculitis (8): both show fibrinoid necrosis of vessel walls and a surrounding inflammatory infiltrate with leukocytoclasia.

In the HgCl₂ model the humoral response and glomerulonephropathy (days 12 to 20) are T cell dependent (9, 10), and the accompanying increase in serum IgE concentration and tissue IL4 mRNA (7, 11) suggests that late tissue injury arises in the context of TH2 cell activation. The mAb R73 (anti-αβ TCR) (12) has been shown to prevent the onset of arthritis (days 12 to 20) in this model (13); however, the pathogenic mechanisms leading to early or late vasculitis are unknown and a direct role for T cells has not been established.

The aims of this work were threefold: 1) to determine the susceptibility of various strains of rat to both the early and late vasculitis, because this tissue injury is unusual in demonstrating a bimodal time course; 2) to examine the role of the αβ T cell in the pathogenesis of both phases of the vasculitis, by αβ T cell depletion using the mAb R73; 3) to test the hypothesis that the mast cell is involved in the pathogenesis of the early vasculitis on the basis that it is abundant within the rat gut, has a perivascular distribution, and is unique in storing preformed proteases and cytokines such as TNF-α (14, 15). Evidence for in vivo mast cell degranulation following HgCl₂ treatment has been sought by measuring serum rat mast cell protease II. A direct role for mast cells in the early vasculitis has been examined by inhibiting mast cell secretory responses in two different ways: 1) use of the drug Doxantrazole (DOX), which, in contrast to other agents such as sodium cromoglycate and ketotifen, stabilizes both populations of mast cells in the rat (16, 17); and 2) use of the mAb G63 (18, 19).
### Materials and Methods

**Animals, Abs, and chemicals**

BN (inbred: RT1<sup>+</sup>), agouti (DA, inbred: RT1<sup>−/−</sup>), Lewis (LEW, inbred: RT1<sup>+</sup>), Sprague Dawley (SD, outbred), and Wistar Albino Glaxo (WAG, inbred: RT1<sup>+</sup>) rats (150 to 400 g) were obtained from Harlan/Olac (Bicester, U.K.), given food and water ad libitum, and used in age- and sex-matched groups. All experimental procedures were performed under halothane anesthesia.

The R73 (mouse IgG1) and OX19 (IgG1 anti-CDS mAb, pan-T cell Ag)-producing cell lines were obtained from the European Collection of Animal Cell Cultures. An ammonium sulfate cut was made from tissue mast cell (CTMC) secretory response (20). It has also been shown to inhibit ischemia-reperfusion-induced rises in plasma rat mast cell protease II (RMCPII), MPO activity, and epithelial permeability in rats (16). G63 is a mouse IgG1 mAb that recognizes a membrane Ag on both rat MMC and CTMC and partially inhibits FceR1-mediated secretion from RBL cells and rat CTMC (18, 19).

**Vasculitis score**

Early (36–96 h) and late (day 15–17) vasculitis was scored at necropsy by an observer blinded to treatment groups. The macroscopic appearances of the cecal serosa and mucosa were scored separately by a system that has previously been shown to correlate well with histologic scores from directed cecal biopsies (21). The total vasculitis score was the sum of these two macroscopic scores for each animal. In some experiments, histologic vasculitis scores from directed cecal biopsies, stained with hematoxylin and eosin by standard procedures, were recorded by an experienced pathologist blinded to treatment group.

**Strain susceptibility to early and late vasculitis: experiments 1 and 2**

In experiment 1, late vasculitis was studied in DA (n = 6), LEW (n = 6), SD (n = 6), and WAG (n = 7) rats. In experiment 2, early vasculitis was studied in BN (n = 5), DA (n = 4), LEW (n = 6), SD (n = 4), and WAG (n = 4) rats. The experimental protocols are shown in Table I. In experiment 1, serum samples taken throughout the time course were measured for total IgE and anti-MPO Abs (13).

**Role of the αβ T cell in early and late vasculitis: experiments 3, 4, and 5**

**Late vasculitis.** The effect of R73 mAb treatment on HgCl<sub>2</sub>-induced late vasculitis was examined in experiment 3. BN rats received HgCl<sub>2</sub> + R73 (n = 10), HgCl<sub>2</sub> alone (n = 5), HgCl<sub>2</sub> + MOPC-21 (n = 5), or R73 alone (n = 5). The experimental protocols are shown in Table I. R73 and MOPC-21 were injected i.p., 70 μg in 1 ml of PBS per injection.

**Early vasculitis.** The effect of R73 on early vasculitis in BN rats was examined in experiments 4 and 5. The same group sizes were used as in experiment 3, and the experimental protocols are shown in Table I. In experiment 4, animals were treated with R73 for 3 days before the start of HgCl<sub>2</sub> treatment, and in experiment 5 they received R73 for 17 days before the start of HgCl<sub>2</sub>. Therefore when vasculitis was scored in experiment 4 (late vasculitis) and in experiment 5 (early vasculitis), animals had received R73 for the same duration of time.

**Animals were bled from the tail artery into preheparinized syringes (1000 U/ml), and plasma was stored at −20°C.** The proportion of OX19<sup>+</sup> peripheral blood lymphocytes was determined by FACS analysis (13) in all animals every 3 to 4 days throughout each experiment. Total plasma IgE and anti-MPO Abs were measured by ELISA (13) at each of these time points in experiment 3.

**Serum RMCPII analysis following in vivo HgCl<sub>2</sub> treatment:** experiment 6

BN rats (n = 5) received a single injection of HgCl<sub>2</sub> at time 0 and were bled (0.2 μl) from the tail artery at 10 time points during the next 48 h. Serum was stored at −20°C, and the RMCPII concentration was measured by ELISA using a kit purchased from Morredun Animal Research Institute, Edinburgh, U.K. (used according to the manufacturer’s instructions).

**Role of the mast cell in early vasculitis: experiments 7, 8, and 9**

The effect on HgCl<sub>2</sub>-induced early vasculitis of the mast cell secretion inhibitory agents DOX and G63 were studied in experiments 7, 8, and 9. The experimental protocols are shown in Table I. Blood samples were taken from all animals at several time points for serum RMCPII analysis.

**DOX treatment.** In experiment 7, BN rats received HgCl<sub>2</sub> + NaHCO<sub>3</sub> (n = 10), HgCl<sub>2</sub> + DOX (n = 10), DOX alone (n = 5), or NaHCO<sub>3</sub> alone (n = 5); and in experiment 8, they received either HgCl<sub>2</sub> + PBS (n = 9) or HgCl<sub>2</sub> + DOX (n = 9). DOX was injected as a 0.3% solution in 0.1 M NaHCO<sub>3</sub> (30 mg/kg i.p. per injection).

**G63 treatment.** In experiment 9 BN rats received HgCl<sub>2</sub> alone (n = 5), HgCl<sub>2</sub> + MOPC-21 (n = 5), or HgCl<sub>2</sub> + G63 (n = 10). G63 and MOPC-21 were injected into the tail vein (50 μg in 0.5 ml of PBS per injection).

**Statistical analysis**

Data were analyzed using the computer program Instat 2 version 2.04a (GraphPad Software). All statistical analyses were performed using two-tailed probability criteria, unless explicitly stated otherwise. The repeated measures test (repeated measures) was performed by calculating for each animal the sum of scores at repeated time points and comparing the resulting median sum scores of different groups with the Mann-Whitney U test.

### Results

**Strain susceptibility to early and late vasculitis**

**Experiment 1 (late vasculitis).** None of the animals (DA, LEW, SD, WAG) developed macroscopic or histologic signs of tissue injury, or of the humoral response (IgE and anti-MPO Abs), characteristic of HgCl<sub>2</sub>-induced autoimmunity in BN rats.

**Experiment 2 (early vasculitis).** At least one animal in every strain developed early vasculitis. The incidence and the mean total vasculitis scores were respectively: BN 4 of 5, 4.8; DA 2 of 4, 1.25; LEW 5 of 6, 2.8; SD 1 of 4, 0.75; WAG 4 of 4, 3. The presence of vasculitis was confirmed in each strain by histologic examination of directed cecal biopsies (data not shown).

**Effect of R73 on peripheral blood OX19<sup>+</sup> lymphocytes and T cell function**

**Experiments 3 to 5.** R73 treatment induced a fall in OX19<sup>+</sup> PBL from ~50% of total PBL to ~10 to 14% after 3 days of treatment; this was maintained for the duration of each experiment (data not shown). In experiment 3, animals treated with HgCl<sub>2</sub> + MOPC-21 showed a rise in total IgE and in IgG anti-MPO Abs between days 8 and 17. There was no such rise in animals receiving HgCl<sub>2</sub> + MOPC-21.
The document discusses experiments involving HgCl₂ treatment in BN rats to study the role of T cells in vasculitis. It includes graphs showing mean plasma anti-MPO titer and mean plasma total IgE concentration, tables detailing the incidence and severity of HgCl₂-induced early and late vasculitis, and a discussion on the role of the αβ T cell in early and late vasculitis.

**Role of the αβ T cell in early and late vasculitis**

**Experiment 3 (late vasculitis).** One animal (HgCl₂ alone) died at day 15. There was no difference in the incidence or severity of late vasculitis at day 17 in animals treated with HgCl₂ alone or HgCl₂ + MOPC-21. In contrast, none of the animals treated with HgCl₂ + R73, or R73 alone, developed vasculitis (see Table II and Fig. 2). Histologic vasculitis scores from directed cecal biopsies in experiment 4 confirmed the macroscopic findings; median score HgCl₂ ± MOPC-21 = 1, HgCl₂ + R73 = 3 (NS, Mann-Whitney U test).

**Peripheral blood RMCP II concentration following HgCl₂ treatment**

**Experiment 6.** The serum concentration of RMCP II rose in all 5 animals within 12 h of one injection of HgCl₂ (illustrated in Fig. 4). Serum RMCP II concentrations have also been measured at less frequent intervals in a total of 76 BN rats between 6 and 72 h after treatment with HgCl₂ ± PBS, MOPC-21, or NaHCO₃ (including experiments 6, 7, 8, and 9). In 26 animals (34%), the concentration rose to >50% of baseline on at least one of the time points sampled.
The pathogenesis of late vasculitis has similarities with other features of this autoimmune model, in that HgCl₂-induced arthritis is also αβ T cell dependent (13), and the glomerular disease and humoral response are both T cell dependent (9, 10). The peripheral blood αβ T cell population is composed of both CD4 and CD8 T cells. In view of the recent report that late vasculitis is unaffected by anti-CD8 treatment (22), it is likely that CD4 T cells are responsible, at least in part, for the pathogenic role of the αβ T cell population. This is consistent with the cytokine and humoral response following HgCl₂ treatment which suggests Th2 cell activation (7, 11).

Three other animal models provide direct evidence that T cells may play a role in the pathogenesis of vasculitis. In pigs, cytolytic T cells respond to a transfected human class I Ag in the arterial wall and induce a focal granulomatous vasculitis (23). The cell transfer of microvascular smooth muscle sensitized lymphocytes results in vasculitic injury in BALB/c (24) and MRL-+/+ (25) mice. Therefore, taken together with late vasculitis in HgCl₂-induced autoimmunity, animal models demonstrate in three different species that T cells are capable of inducing a pathologic response that results in vasculitis.

In human vasculitis, evidence that T cells have a pathogenic role is indirect and in part derived from the variable finding of lymphocytes within the inflamed vessel wall (2). Elevated concentrations of serum soluble IL-2 receptor suggesting T cell activation (26) also support a role for T cells; however, a close correlation with disease activity has not always been found (27). Supportive evidence also comes from reports of the successful treatment of vasculitis with anti-T cell mAbs and anti-thymocyte globulin (28).

The presence of pathogenic T cells is suggested by a small but significant increase in the number of T cell clones expressing Vα2.1 in the peripheral blood of patients with vasculitis (29), but in vitro searches for T cells with autoreactivity to postulated vasculitis Ags have given equivocal results (30, 31). Because MHC molecules are involved in the presentation of peptides to T cells, a positive association of particular MHC alleles with disease would also support a role for T cells in pathogenesis. However, two large studies have failed to detect such an association, although one did find a protective association with HLA-DR13/Dw6 (32, 33).

In the HgCl₂ model, only susceptible strains (RT1⁺) develop late vasculitis and the other humoral and tissue manifestations of this syndrome (4). In contrast, early vasculitis appears to be under separate control in not demonstrating strain susceptibility. Furthermore, R73 treatment had no effect on the early vasculitis despite the use, in experiment 5, of the same dose and duration of R73 that had inhibited T cell function and completely abolished the late vasculitis. This indicates that early vasculitis (which is histologically indistinguishable from late vasculitis) results from separate non-αβ T cell mechanisms. The finding that IL-4 mRNA is also up-regulated in BN cecal tissue at the time of early vasculitis (7) may be relevant to the pathogenesis of this early αβ T cell-independent tissue injury. The mast cell is both a potential source of IL-4 (34) and a potential mediator of a local acute inflammatory response. There are two anatomically and functionally separate populations of mast cells in the rat; MMC are restricted in the bowel to the mucosa, and CTMC are more widespread, and in the bowel they are found in the submucosa, serosa, and peritoneal cavity (35). Both populations have a perivascular distribution; and in addition to IL-4 secretion, they store and secrete numerous cytokines, prostaglandins, leukotrienes, and hydrolytic enzymes (35-37). A potential pathogenic role in rat intestinal pathology is supported by the finding that infusion of RMCP II into the mesenteric artery of rat jejunum results in an increase in mucosal permeability (38). In humans, the role of mast cells in systemic vasculitides is...
Evidence that mast cells may be specifically involved in the pathogenesis of HgCl₂-induced autoimmunity comes from the finding that nontoxic concentrations of HgCl₂ sensitize BN rat mast cells for mediator release and cause up-regulation of mast cell IL-4 mRNA (41). These findings demonstrate that HgCl₂ has the potential to reduce the threshold for proinflammatory mediator release and induce IL-4 transcription in vivo in BN mast cells. Further evidence that the in vivo syndrome is not due to nonspecific toxic effects of HgCl₂ is that gold compounds and D-penicillamine, both of which cause autoimmunity in the BN rat (42, 43), produce an identical histologic lesion with necrotizing vasculitis (44).

RMCP II is secreted by MMC (but not CTMC) following activation and degranulation (45). In BN rats, a single injection of HgCl₂ was followed in experiment 6 by a rise in serum RMCP II at 12 h, indicating in vivo MMC degranulation temporally before the onset of early vasculitis. In a larger series of animals, less frequent blood samples were taken; however, a similar rise in serum RMCP II was also found in 34% of animals between 12 and 72 h after HgCl₂. This is likely to be an underestimation because only a large increase in intestinal RMCP II is detected by a rise in serum RMCP II (16). The association among HgCl₂ treatment, a rise in serum RMCP II, and early vasculitis is strengthened by the finding that DOX and G63 treatment resulted in a significantly lower proportion of animals with a detectable rise in serum RMCP II. This suggests that these two agents had an inhibitory effect in vivo on the secretory activity of the MMC population.

Attempts to study the effects of inhibitors of mast cell secretory responses on the early vasculitis were confounded by considerable interexperiment variation in the incidence and severity of vasculitis in the control groups. For this reason, animals were studied at different times (24-96 h) after the start of HgCl₂ treatment in a number of experiments. In every case, the results indicated a consistent reduction in the incidence and severity of early vasculitis in the DOX and G63 groups; experiments 7, 8, and 9 are representative examples of this work. The lack of complete abolition of tissue injury may be an indication that other cells are involved in the pathogenesis of the early vasculitis. However, the design of these experiments was compromised by the lack of previous data regarding the in vivo pharmacokinetics of both DOX and G63, and

TABLE III. Incidence and severity of HgCl₂-induced early vasculitis: experiments 7, 8, and 9

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Incidence (%)</th>
<th>Severity (Median Total Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgCl₂ + PBS</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>HgCl₂ + PBS</td>
<td>60 (p = 0.045)</td>
<td>2 (p = 0.035)</td>
</tr>
<tr>
<td>HgCl₂ + PBS</td>
<td>66 (NS)</td>
<td>2 (p = 0.012)</td>
</tr>
<tr>
<td>HgCl₂ + PBS</td>
<td>50 (NS)</td>
<td>0 (p = 0.05)</td>
</tr>
<tr>
<td>HgCl₂ + PBS</td>
<td>20 (NS)</td>
<td>0 (p = 0.05)</td>
</tr>
</tbody>
</table>

*vs. HgCl₂ + NaHCO₃, PBS, MOPC-21, one-tailed Fisher's exact test.
**vs. HgCl₂ + NaHCO₃, PBS, MOPC-21, one-tailed Mann-Whitney U test.
it is possible that the treatment protocols were insufficient to completely inhibit mast cell activation. This is suggested in experiment 8 where the injection of DOX at shorter time intervals than in experiment 7 resulted in a greater protective effect. This result complements the significant reduction in the number of animals in the HgCl₂ + DOX group with a rise in serum RMCP II and suggests that mast cells are involved, at least in part, in the pathogenesis of early vasculitis in this model.

There are possible analogies between our findings in the BN rat and work on the initiation of delayed type hypersensitivity in the mouse. In this latter system, release of mediators (probably mainly serotonin) from either mast cells (46) or platelets (47) initiates a rapid inflammatory response. This is followed by a slower T cell-mediated response which is dependent on the initial response. It is a speculative possibility that in our model, as well as mediating the early inflammatory (vasculitis) response, mast cells via the production of IL-4 could play a role in initiating and/or augmenting the subsequent Th2 cell-dependent inflammatory response.

In conclusion, these findings suggest that early and late vasculitis in the HgCl₂ model are controlled by different cellular and genetic mechanisms. The finding of an αβ T cell-dependent phase supports previous data from other animal models of vasculitis and indirect evidence in humans that T cells have an important role in the pathogenesis of vasculitis. The HgCl₂ model is unique in demonstrating an αβ T cell-independent phase in the pathogenesis of the same histologic lesion. Evidence is presented that MMC degranulate following in vivo HgCl₂ treatment and that mast cells play a role in the pathogenesis of early vasculitis. This emphasizes the potential contribution of these cells to local tissue injury and raises the possibility that mast cells may be important in the subsequent polarization of the immune response to the Th2 type in HgCl₂-induced autoimmunity.

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References


