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Histamine ameliorates anti-glomerular basement membrane antibody-induced glomerulonephritis in rats

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Anti-glomerular basement membrane (anti-GBM)-induced glomerulonephritis involves T-helper type 1 (Th1) responses leading to rapid crescent formation. As many inflammatory and immune responses in general are affected by histamine, we examined the effects of histaminergic ligands on immune renal injury in the rat. Female Wistar-Kyoto rats were injected intraperitoneally with an antibody against the GBMs. Histaminergic ligands were then injected twice daily for 5 days after which renal function was assessed by proteinuria. Treatment with histamine led to significant dose-dependent reductions in proteinuria compared to the control antibody-injected group and markedly decreased the number of crescentic glomeruli and macrophage infiltration of the glomeruli. Furthermore, histamine significantly decreased the plasma concentration of interleukin-12, a Th1-type cytokine compared to the antibody-injected control animals. Dimaprit, an H_2/H_4 agonist, mimicked the effects of histamine on proteinuria and crescent formation. Clozapine, an H_4 agonist, tended to mimic the effects of histamine, whereas an H_1, mepyramine, or an H_2 antagonist, ranitidine, did not reverse the protective effect of histamine. We suggest that histamine may alleviate renal injury in anti-GBM glomerulonephritis by suppressing the immune response.

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Anti-glomerular basement membrane (anti-GBM) glomerulonephritis progresses with crescent formation rapidly, and T-helper type 1 (Th1) responses are closely related to the pathogenesis and progression of this disease.[1–4] The infiltration of inflammatory cells, such as macrophages and T lymphocytes, is a predominant factor in the development of anti-GBM glomerulonephritis. In contrast, T-helper type 2 (Th2) cytokines, such as interleukin (IL)-4 and IL-10, have been reported to attenuate the development of anti-GBM glomerulonephritis in mice by preventing production and function of Th1 cytokines.[4–7]

Histamine is stored within secretory granules of mast cells and basophils. The release of histamine, which is initiated in response to specific antigens, causes an increase in capillary permeability and edema formation. These inflammatory responses are mediated predominantly by histamine H_1 receptors.[8] Conversely, histamine H_2 receptor stimulation has been shown to suppress immune responses by reducing the chemotactic responsiveness of leukocytes and inflammatory cytokines.[9] Whereas histamine H_3 receptors are expressed primarily in the brain, histamine H_4 receptors are expressed in peripheral tissues, particularly, those of hematopoietic lineage, such as bone marrow, spleen, thymus, and leukocytes.[10] Histamine H_4 receptors are also involved in the regulation of immune responses and in inflammatory cell recruitment.[11] Thus, it is likely that histaminergic ligands exert an influence on the development of glomerular immune injury.

In this study, we employed an animal model of anti-GBM glomerulonephritis induced by an intraperitoneal injection of a monoclonal antibody directed against the NC1 domain of type IV collagen. This animal model is a passive antibody-dependent model of anti-GBM glomerulonephritis and different from that of accelerated anti-GBM glomerulonephritis, which depends on active immune responses to deposited heterologous antibodies specific for the GBM. However, passive transfer of the anti-GBM antibody has been shown to induce glomerulonephritis similar to that seen in actively immunized rats, although the magnitude of damage

is less severe.[12] Using this animal model, we evaluated the effects of histaminergic ligands on functional and morphologic outcomes.

RESULTS

Effects of histamine

No animal died during the 6-day period between administration of anti-GBM antibody and killing. Systolic blood pressure did not differ significantly among groups 6 days after antibody injection, and no marked difference was found in the plasma levels of blood urea nitrogen and creatinine (Table 1 and Figure 1).

Endocapillary proliferation, severe fibrinoid necrosis, and marked crescent formation were identified 6 days after antibody injection (Figure 2a). Histamine decreased the number of crescent glomeruli in a dose-dependent manner (Figure 2b and c). The percentages of crescentic glomeruli in the histamine (20 mg/kg)-treated and histamine (50 mg/kg)-treated groups were 47 and 15%, respectively, of that in the antibody-injected control group.

Immunofluorescence showed linear deposition of antibody along the GBM in animals injected with antibody (Figure 3). Likewise, antibody deposition was observed in animals treated with histamine twice a day (20 mg/kg, each). The intensity of immunofluorescence was similar between the two groups. On the other hand, no deposition of complement C3 was shown in glomeruli in either the antibody-injected or histamine-treated group.

Immunohistochemistry showed no ED-1-positive cells (rat macrophages) in glomeruli of intact animals. Injection of anti-GBM antibody recruited macrophages to glomeruli (Figure 4a), and the number of macrophages in 50 glomeruli was 367 ± 101 (mean $\pm$ s.d., $n = 7$). Treatments with histamine decreased the number of macrophages (Figure 4b). The number of macrophages in 50 glomeruli in the histamine (20 mg/kg)-treated group was 217 ± 63 ($n = 5$) ($P < 0.05$).

Table 1 | Laboratory data and systolic blood pressure

	Blood urea nitrogen (mg/dl)	Creatinine (mg/dl)	Blood pressure (mg/dl)
Intact group	11.9 ± 1.1	0.20 ± 0.02	102 ± 6
Antibody-injected group	13.2 ± 3.0	0.20 ± 0.01	110 ± 11
Histamine-treated antibody-injected group	11.5 ± 2.4	0.19 ± 0.01	112 ± 12

GBM, glomerular basement membrane.
Following the measurement of blood pressure 6 days after anti-GBM antibody injection, blood was collected and analyzed with routine laboratory procedures. Values represent the mean $\pm$ s.d. of five animals.

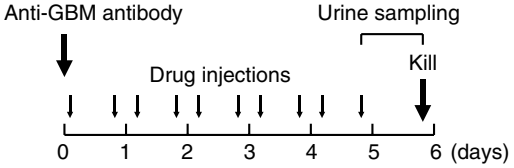

Figure 1 | Experimental procedures. After an intraperitoneal injection of anti-GBM antibody, saline or histaminergic ligands were subcutaneously administered twice a day for 5 days. Urine samples for 24 h were collected 5–6 days after antibody injection. Then, kidneys were dissected for histology and immunohistochemistry.

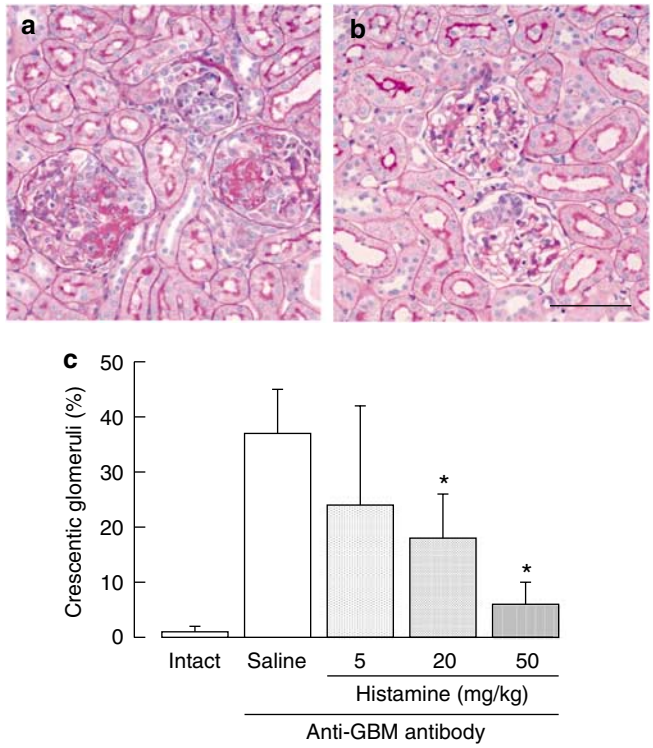

Figure 2 | Representative photomicrographs of periodic acid-Schiff stain and determination of the percentage of crescentic glomeruli. (**a**) Six days after injection of anti-GBM antibody, endocapillary proliferation, severe necrosis, and marked crescent formation were seen in glomeruli. (**b**) In glomeruli from an animal treated with histamine (20 mg/kg) twice a day, endocapillary proliferation was segmental and mild, and crescent formation was rare. (**c**) The percentage of crescentic glomeruli was determined 6 days after antibody injection. Values represent the mean $\pm$ s.d. of 5–8 animals. $^{*}P < 0.01$ as compared to the antibody-injected control group. Bar = 100 μm.

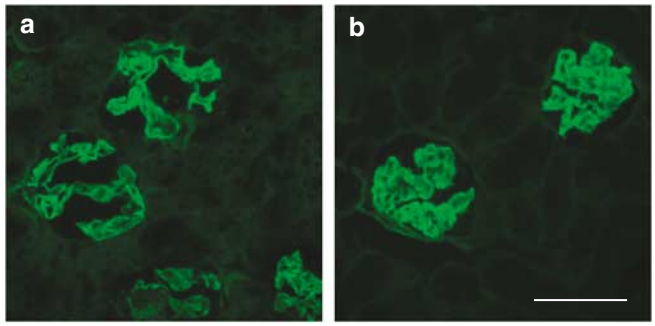

Figure 3 | Representative photomicrographs of direct immunofluorescence of fluorescein isothiocyanate-conjugated anti-rat IgG. (**a**) Six days after injection of anti-GBM antibody, linear deposition of antibody along the GBM was observed in glomeruli. (**b**) In glomeruli from an animal treated with histamine (20 mg/kg) twice a day, deposition of antibody was similar to that in antibody-injected control glomeruli. Bar = 100 μm.