Histamine Receptor Bearing Lymphocytes in Dogs
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It is well known that the histamine inhibits lymphokine production [6], phytohemagglutinine- and concanavalin A-induced lymphocyte proliferation [1, 2, 4], and cutaneous delayed hypersensitivity [6]. These inhibitory effects develop through membrane receptor systems, mainly via H-2 receptor on lymphocytes [4]. The number of histamine receptor bearing lymphocytes in peripheral blood was determined in relation to the immunological states in various human diseases, since the histamine activates suppressor T cells [7]. However, there is a sparse information for these lymphocytes in dogs.

For the detection of histamine receptors, the histamine rosette technique [5] was indicated to be a simple and sensitive method on human and murine lymphocytes [5, 8]. By this method, therefore, we examined the histamine receptor on canine lymphocytes.

The peripheral lymphocytes suspension (5×10⁶ cells/ml) was mixed with equal volume of sheep red blood cells coupled with histamine-porcine albumin conjugate (H-PSA-SRBC, 2.5×10⁶ cells/ml) in a plastic tube, centrifuged at 1000 rpm for 7 min at 4°C, and incubated for 30 min in ice bath. After that, the cell pellets were gently resuspended and stained with 0.05% brilliant cresyl blue. The cells stained were examined under the microscope with a hemocytometer and the percentage of rosette forming cells bounded with three or more H-PSA-SRBC were counted in 200 random lymphocytes. This rosette formation was almost completely inhibited by pretreatment of lymphocytes with H-PSA.

The percentages of histamine receptor-bearing lymphocytes from peripheral blood of 5 healthy beagle dogs were obtained ranging between 12.9% and 23.2% (17.9±3.4%, mean±SD), similarly to those reported in human [9] and murine [5] lymphocytes.

Histamine receptors detected by this assay were analysed by histamine rosette formation inhibition test with H-1 receptor and H-2 receptor agonist or antagonist.

For the inhibition of rosette formation, 0.2ml of lymphocytes suspension (2×10⁸ cells/ml) was mixed with 0.1ml of histamine-dihydrochloride, 2-pyridylethylamine (H-1 receptor agonist), dimaprit (H-2 receptor agonist), or cimetidine (H-2 receptor antagonist) at concentrations from 10⁻⁸M to 10⁻²M in phosphate buffer saline (0.01M phosphate buffer, pH 7.2, 0.14M NaCl) and incubated for 10 min at room temperature. The pretreated lymphocytes were examined for rosette formation by the same procedure described above.

These compounds inhibited the binding of H-PSA-SRBC to lymphocytes (Fig. 1). There was no difference in the degree of inhibition by

Fig. 1. Inhibition of rosette formation with canine blood lymphocyte by histamine-dihydrochloride (●—●), 2-pyridylethylamine (Δ—Δ), dimaprit (○—○), and cimetidine (■—■).
histamine-dihydrochloride, 2-pyridylethylamine, and cimetidine at various concentrations from $10^{-5}$M to $10^{-3}$M, while the dimaprit had a remarkable inhibitory effect on histamine rosette formation. From these results, histamine receptors detected by this assay were supposed to be related with both H-1 and H-2 receptors. The reason for a remarkable inhibitory effect on rosette formation by dimaprit compared with another compounds was not explained clearly. However, the dimaprit might have a different affinity or mechanism to bind the histamine receptor on lymphocytes [1, 3]. Further studies were necessary to understand the biological and physiological function of histamine receptor-bearing lymphocytes in dogs.

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