

Effect of Histamine and Histamine Antagonists on Natural and Antibody-Dependent Cellular Cytotoxicity of Human Lymphocytes *in Vitro*¹

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The *in vitro* effect of histamine and its antagonists, cimetidine and clemastine fumarate, on natural killer (NK) and antibody-dependent cellular cytotoxicity (ADCC) activities of human lymphocytes was investigated. The histamine 1 (H1) antagonist, clemastine fumarate, and the histamine 2 (H2) antagonist, cimetidine, but not histamine alone, inhibited the NK and ADCC activities of lymphocytes when added directly to the mixture of effector and target cells in a ⁵¹Cr-release assay. This inhibition was proportional to the concentration of drugs added and was observed at various effector to target ratios against several targets. H1 and H2 antagonists also inhibited NK activities of T cells as well as Percoll-separated, NK-enriched effector cells. The inhibition was significantly reversed by histamine. In target binding assays, clemastine fumarate and cimetidine also decreased the target binding capacity of effector lymphocytes. Further, PBL precultured with histamine (10^{-3} - 10^{-4} M) for 24 hr showed a significant decrease in their NK and ADCC activities. In coculture experiments, PBL precultured with histamine suppressed the NK activity of normal autologous effector lymphocytes. PBL precultured with histamine showed an increased number of OKT8⁺ cells, as estimated using monoclonal antibodies. The suppression of cytotoxicity was not due to either direct toxicity, steric hindrance, crowding, or cell death, but by functionally viable suppressor cells. An immunoregulatory role for histamine in NK and ADCC reactions is proposed.

INTRODUCTION

We and our co-workers have previously demonstrated that unstimulated, cultured human peripheral blood lymphocytes (PBL)⁴ can develop suppressor cell activities against lymphocyte transformation, polyclonal B-cell activation, and natural killer (NK) and antibody-dependent cellular cytotoxicity (ADCC) reactions, as a possible consequence of an autologous mixed-lymphocyte culture reaction (1-3). We have also shown that serum-free culture supernatants of unstimulated PBL contain soluble

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⁴ Abbreviations used: ADCC, antibody-dependent cellular cytotoxicity; CEM, human T-lymphoblastoid cell line; E:T, effector to target ratio; H1, histamine 1 receptor; H2, histamine 2 receptor; K562, human erythroleukemia cell line; NK, natural killer; PBL, peripheral blood lymphocytes depleted of adherent cells; SB, human B-leukemia cell line; TBC, target binding cells; T γ , T cells with Fc receptors for IgG.

suppressor factor(s) (SSF) that significantly exhibit suppressor activity toward mitogenesis and alloantigen stimulation in mixed-lymphocyte culture (4), and NK and ADCC reactions (5). Other studies on T-lymphocyte subpopulations have shown that T cells bearing Fc receptors for human IgG (T γ) mediate both NK and ADCC activities (6, 7) and exhibit suppressive effects on various lymphocyte reactions (8–10).

The role of histamine, a vasoactive amine, in immunoregulation has been extensively studied. *In vitro* investigations showed that histamine inhibits cell-mediated cytotoxicity, lymphocyte proliferative responses to mitogens and soluble antigens, lymphokine production, and the mixed-lymphocyte reaction (11–15). Our interest in immunoregulation of natural and antibody-dependent cellular cytotoxicity of human lymphocytes led us to investigate the effect of histamine and histamine antagonists on NK and ADCC activities of human lymphocytes. The results indicate that lymphocytes precultured with histamine demonstrated a significant decrease in their NK and ADCC activities and were able to suppress the NK activity of autologous effector cells in coculture experiments. An immunoregulatory role of histamine in cellular cytotoxic reactions is thus proposed.

MATERIALS AND METHODS

Drugs. The H1 receptor blocker, clemastine fumarate (Tavist, Dorsey Labs, Lincoln, Nebr.), was used in a concentration range of 1×10^{-8} to 1×10^{-5} M; whereas the H2 receptor blocker, cimetidine (Smith-Kline and French Labs, Philadelphia, Pa.), was used in a concentration range of 1×10^{-4} to 2.5×10^{-2} M. Histamine (Sigma Chemical Co., St. Louis, Mo.) was used in concentrations of 1×10^{-5} to 1×10^{-2} M.

Experimental design. Drugs were added directly to the mixture of effector and target cells in a 4-hr ^{51}Cr -release assay. Controls containing either target and effector cells, or targets and drugs, were run concomitantly. In separate experiments, lymphocytes were also precultured with histamine for 0 to 24 hr, washed, and tested for NK and ADCC activities or used in mixing experiments to measure their suppressive effects on autologous lymphocytes.

Monoclonal antibodies. OKT3, OKT4, and OKT8 antibodies were purchased from the Ortho Pharmaceutical Corporation, Raritan, New Jersey and were used in a final dilution of 1:100. These antibodies are complement (C) fixing and of the IgG isotype.

Lymphocyte donors. Peripheral blood from adult healthy donors of both sexes was drawn into a plastic syringe containing heparin (20 U/ml). Donors were apprised of the study and consents were obtained consistent with the policies of The University of Michigan and the National Institutes of Health.

Isolation of lymphocytes. PBL were isolated from heparinized venous blood using a modified method of Böyum (16). Blood was diluted with an equal volume of normal saline and centrifuged at 400g for 30 min at 18°C. The mononuclear cell band was harvested, washed three times with saline, and resuspended in RPMI 1640 medium, containing 25 mM HEPES buffer supplemented with 5% heat-inactivated fetal calf serum (FCS) (GIBCO, Grand Island, N.Y.), 80 $\mu\text{g}/\text{ml}$ gentamicin (Shering Corp., Kenilworth, N.J.), and 300 μg fresh glutamine/ml (complete medium).

Depletion of adherent cells. Mononuclear cells were depleted of adherent cells by passing through a G-10 column. PBL were suspended in RPMI 1640 with 10% FCS

and passaged through a 7-ml column of Sephadex G-10 beads (Pharmacia Fine Chemicals, Piscataway, N.J.), equilibrated in the same medium. After 45 min of incubation at 37°C, nonadherent cells were washed through with one bed volume of warm (37°C) medium. The cell recovery was >70% of the total input, and macrophage contamination as indicated by nonspecific esterase staining was <2%.

Preparation of T cells. T cells were prepared as described elsewhere (17). Briefly, 4×10^6 PBL depleted of adherent cells were mixed with 0.25 ml of heat-inactivated FCS preabsorbed with sheep red blood cells (SRBC) and 1 ml of 1% packed volume, neuraminidase-treated (25 U/ml in a 5% SRBC suspension) SRBC. The mixture was incubated at 37°C for 5 min and centrifuged at 200g for 5 min followed by incubation at 4°C for 1 hr. The pellets were gently resuspended and T cells rosetted with SRBC were separated from non-T lymphocytes on a Ficoll-Hypaque (Pharmacia) density gradient by centrifugation at 480g for 20 min at 20°C. SRBC attached to the isolated T lymphocytes (pellet) were lysed by hypotonic treatment with 1 ml of distilled water, followed by 1 ml of double-strength minimal essential medium (MEM, GIBCO). T cells were washed three times with RPMI 1640 and resuspended in complete medium and preincubated for 24 hr before use.

Percoll fractionation of lymphocytes. The enrichment of NK effector cells using a discontinuous gradient of Percoll (Pharmacia) was carried out as described elsewhere (18). To prepare a discontinuous density gradient, Percoll solution was mixed at various concentrations with RPMI 1640 medium, and 2-ml aliquots (ranging from 50.0 to 37.5% in 2.5% increments) were gently layered into 15 × 130-mm round-bottom glass test tubes. Adherent cell-depleted lymphocytes were layered on top of the gradient and centrifuged at 300g for 45 min at 20°C. Six fractions, with the uppermost (37.5%) designated "0" and the lowermost (50.0%) designated "V," were collected from the top with a Pasteur pipet and washed twice in RPMI 1640 medium. The least dense fraction banding at the 37.5% Percoll interface consistently showed the highest NK activity and was used routinely as a source of NK-enriched cells. Recovery of cells was 85% of that input, and viability always exceeded 95% by trypan blue dye exclusion.

In vitro incubation of lymphocytes with histamine. Lymphocytes depleted of adherent cells, T cells, and Percoll-separated NK-enriched effector cells were suspended in complete medium at a concentration of 3×10^6 cells/ml to which histamine was added. A duplicate set of cultures received RPMI 1640 medium instead of histamine as control. Cell cultures were incubated for 0 to 24 hr in a humidified environment of 5% CO₂ in air at 37°C. Cells were washed twice and resuspended in complete medium. Cell viability was comparable with that of control cultures as determined by trypan blue dye exclusion and was not affected by histamine concentrations of $\leq 1 \times 10^{-2}$ M. Treated and control cultures were tested for their ability to mediate NK and ADCC activities.

Tumor target cells. The human erythroleukemia cell line, K562, and T-lymphoblastoid cell line, CEM, were used as targets for NK cells. For the ADCC assay, we chose as targets a human B-cell leukemia line (SB) because of resistance to NK activity in a 4-hr ⁵¹Cr-release assay.

Preparation of target cells. Passaged 48-hr-old target cells were washed twice with complete medium. To 0.8 ml aliquots of complete medium containing 5×10^6 tumor cells, 200 μCi of ⁵¹Cr as sodium chromate (New England Nuclear, Boston, Mass.) was added. The cells were incubated at 37°C for 1 hr in a humidified atmosphere

of 5% CO₂ in air with intermittent shaking. Following incubation, the cells were washed three times with complete medium and resuspended to a concentration of 2×10^5 cells/ml.

Assay for NK activity. NK activity was determined in a direct ⁵¹Cr-release assay as previously described (3, 5, 19, 20). Briefly, varying concentrations of viable effector cells in complete medium were added to triplicate cultures of ⁵¹Cr-labeled target cells in 0.2-ml volumes in V-bottom microtitration plates (Dynatech Labs, Alexandria, Va.). After centrifugation at 40g for 2 min, they were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 4 hr. Percentage cytotoxicity was calculated as

$$\% \text{ cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100,$$

where spontaneous release represents counts released from control wells containing only 2×10^4 target cells, and total release represents counts obtained in an aliquot of 2×10^4 lysed target cells.

Assay for ADCC. The ADCC activity of effector cells was determined by the original method of Perlmann and Perlmann (21) as modified by Handwerker and Koren (22). Briefly, 50 μ l of varying concentrations of effector cells was added to 50 μ l of complete medium containing ⁵¹Cr-labeled SB target cells and 100 μ l of a 2×10^{-4} dilution of rabbit anti-SB antisera previously found to yield maximal ADCC. Percentage ADCC activity was calculated as described above for NK activity with the following exceptions. Spontaneous release represents counts released in control wells containing effector cells, labeled SB target cells, and medium instead of anti-SB antibodies, and total release represents counts obtained in an aliquot of 1×10^4 ⁵¹Cr-labeled SB target cells. Percentage inhibition of NK and ADCC activities by drugs was calculated as

$$\% \text{ inhibition} = \frac{\% \text{ cytotoxicity of control lymphocytes} - \% \text{ cytotoxicity of drug treated lymphocytes}}{\% \text{ cytotoxicity of control lymphocytes}} \times 100.$$

Assay for complement-dependent cytotoxicity. Lymphocytes (5×10^6) were suspended in serum-free RPMI 1640 medium to which monoclonal antibodies were added to yield a final titer of 1:100 (previously found to be optimal). The cultures were incubated at room temperature for 30 min. They were then washed twice with serum-free medium and suspended in a 1:4 final dilution of rabbit complement. The mixtures were incubated for 1 hr at 37°C and washed twice with serum-free medium. The number of positive cells stained with trypan blue was counted, using a hemacytometer.

Target binding assay. Target binding cell (TBC) assay was used as described by Haliotis *et al.* (23). In brief, lymphocytes precultured separately with either of the drugs, or without drugs, were washed twice and mixed with a fivefold excess of tumor cells centrifuged at 150g for 5 min and incubated at 37°C for 5 min. Following incubation, lymphocyte-target mixtures were transferred to ice, the pellets were gently resuspended, and the number of lymphocytes binding to tumor targets was determined, using a hemacytometer.

RESULTS

Effect of Histamine on NK and ADCC Activities of Lymphocytes

In view of the finding that human lymphocytes have histamine receptors, and such lymphocytes exert various immunoregulatory functions in man (11-14, 24-26), the

influence of histamine on NK and ADCC activities of lymphocyte subpopulations was studied. Data presented in Table 1 show that histamine added at different concentrations ranging from 1×10^{-2} to 1×10^{-5} M to the reaction mixture of fresh effector and target cells and incubated for 4 hr showed no significant effect on NK and ADCC activities of either total PBL or isolated T cells.

Effect of Histamine Antagonists on NK and ADCC Activities of Lymphocytes

Table 2 shows a dose-response effect of histamine antagonists, clemastine and cimetidine, on NK and ADCC activities of lymphocytes. Clemastine, at concentrations of $\geq 2.5 \times 10^{-5}$ M, and cimetidine, at concentrations of $\geq 5 \times 10^{-2}$ M, significantly inhibited NK and ADCC activities. Cimetidine at concentrations $< 5 \times 10^{-2}$ M consistently showed detectable suppression of NK and ADCC activities of lymphocytes, although the level of suppression was not significant. Therefore, a concentration of 5×10^{-2} M which was not toxic to effector or target cells was used in subsequent experiments. The results suggest that histamine antagonists inhibit or block NK and ADCC reactions of lymphocytes.

Table 3 shows the inhibition of NK activity of lymphocytes against K562 and CEM targets and ADCC activity against SB targets at different E:T cell ratios by histamine and its antagonists. It is evident that the inhibitory effect of drugs occurs even at higher E:T cell ratios. No significant inhibition was obtained with histamine at any E:T cell ratios.

The effect of histamine and its antagonists on NK and ADCC activities of lymphocyte subpopulations is presented in Table 4. The results demonstrate that H1 and H2 antagonists significantly inhibited NK and ADCC activities of both PBL and T cells. Histamine did not produce any significant effect on NK and ADCC activities of either PBL or T cells.

TABLE 1
Effect of Histamine on NK and ADCC Activities^a

Histamine concentration (M)	Cytotoxicity (%) ^b			
	PBL ^c		T cells ^d	
	NK ^e	ADCC ^f	NK	ADCC
0	50.0 ± 3.4	36.1 ± 5.2	46.2 ± 2.6	34.3 ± 2.5
1×10^{-5}	46.2 ± 5.8	38.2 ± 1.3	39.2 ± 6.2	31.8 ± 2.8
1×10^{-4}	44.7 ± 8.6	35.0 ± 3.0	42.7 ± 2.9	28.6 ± 3.2
1×10^{-3}	47.1 ± 2.4	35.6 ± 5.5	41.3 ± 7.5	30.0 ± 3.2
1×10^{-2}	47.3 ± 1.3	34.4 ± 3.5	41.0 ± 3.2	27.7 ± 4.2

^a Histamine was added directly to the mixture of effector and target cells in a 4-hr ⁵¹Cr-release assay. Results are expressed as means ± SEM of triplicate determinations from a representative experiment and four separate experiments gave similar results.

^b Percentage cytotoxicity was calculated as described under Materials and Methods.

^c PBL depleted of adherent cells as described under Materials and Methods.

^d T cells separated by rosetting as described under Materials and Methods.

^e NK activity was measured against the human erythroleukemia cell line, K562.

^f ADCC activity was measured against the human B-lymphoblast cell line, SB, sensitized with anti-SB sera.

TABLE 2
Dose-Response Effect of Antihistaminics on NK and ADCC Activities^a

Drug	Concentration (M)	Cytotoxicity (%) ^b	
		NK ^c	ADCC ^d
None	—	70.2 ± 2.4	50.8 ± 2.4
Clemastine fumarate	1 × 10 ⁻⁸	74.0 ± 3.2	52.0 ± 2.7
	1 × 10 ⁻⁷	72.0 ± 2.1	49.2 ± 4.2
	5 × 10 ⁻⁷	66.5 ± 1.1	45.7 ± 3.2
	5 × 10 ⁻⁶	63.5 ± 8.4	48.7 ± 4.2
	7.5 × 10 ⁻⁶	64.8 ± 5.3	47.5 ± 2.2
	2.5 × 10 ⁻⁶	38.4 ± 2.3*	37.6 ± 4.3*
	1 × 10 ⁻⁵	9.0 ± 4.3**	10.7 ± 11.8**
Cimetidine	1 × 10 ⁻⁴	72.0 ± 2.8	52.0 ± 2.8
	5 × 10 ⁻⁴	50.9 ± 4.8	46.3 ± 4.3
	7.5 × 10 ⁻³	43.7 ± 2.4	43.3 ± 4.9
	5 × 10 ⁻³	26.2 ± 1.4*	20.8 ± 3.9*
	2.5 × 10 ⁻²	10.2 ± 2.8**	13.7 ± 3.3**

^a Drugs were added directly to the mixture of effector and target cells in a 4-hr assay at a 50:1 E:T ratio.

^b Percentage cytotoxicity was calculated as described under Materials and Methods. Results are expressed as means ± SEM of four experiments.

^c NK activity was measured against the human erythroleukemia cell line, K562.

^d ADCC activity was measured against the human B-lymphoblast cell line, SB, sensitized with anti-SB sera.

* $P < 0.05$.

** $P < 0.01$.

Data presented in Table 5 reveal the effect of drugs on Percoll-separated NK-enriched effector cells. Clemastine and cimetidine significantly inhibited NK activity of enriched effector cells while histamine did not manifest any significant effect on NK activity.

To determine whether the addition of histamine reduces the inhibitory effect of antihistaminics, we added histamine together with antihistaminics to the test system in a 4-hr assay (Table 6). Addition of either clemastine and cimetidine alone to the reaction mixture significantly inhibited NK and ADCC activities of lymphocytes. The inhibitory effect of clemastine and cimetidine was markedly reduced by the simultaneous addition of histamine. The same concentration of histamine (1×10^{-3} M) used alone in the system did not produce any significant effect on either NK or ADCC activity of lymphocytes.

Effect of Histamine Antagonists on Target Binding Cells

To examine whether the suppression of cytotoxicity of lymphocytes by histamine antagonists is due to interference with a histamine receptor-associated target recognition structure rather than inhibition of the lytic mechanism, target binding assays were performed. The results of such studies are presented in Table 7. Clemastine (2.5×10^{-5} M) and cimetidine (5×10^{-2} M), at NK and ADCC inhibitory concentrations, significantly reduced the percentage of target binding lymphocytes (19 and 13%, respectively) compared to control lymphocytes (37%). Histamine at either 1×10^{-3} or 1×10^{-7} M concentrations did not affect the target binding ability of lymphocytes.

TABLE 3
Effect of Antihistaminics on NK and ADCC Activities of Lymphocytes at Various E:T Cell Ratios^a

Target cell	Drug	Concentration (M)	E:T cell ratio ^b					
			100:1		50:1		20:1	
			Cytotoxicity (%) ^c	Inhibition (%) ^c	Cytotoxicity (%)	Inhibition (%)	Cytotoxicity (%)	Inhibition (%)
K562 (NK) ^d	—	—	38.0 ± 4.1	—	24.9 ± 2.4	—	11.2 ± 1.8	—
	Clemastine	2.5 × 10 ⁻⁵	12.4 ± 2.1	67.3	9.2 ± 0.8	63.0	4.4 ± 0.4	60.7
	Cimetidine	5 × 10 ⁻²	9.7 ± 1.2	74.4	6.6 ± 0.7	73.4	3.7 ± 0.3	66.9
CEM (NK) ^f	Histamine	1 × 10 ⁻³	34.9 ± 3.4	8.1	27.7 ± 2.4	-11.2 ^e	15.2 ± 1.8	-35.7
	—	—	18.0 ± 2.2	—	7.5 ± 0.6	—	4.3 ± 0.9	—
	Clemastine	2.5 × 10 ⁻⁵	4.8 ± 0.5	73.3	2.4 ± 0.1	68.0	1.8 ± 0.1	58.1
SB (ADCC) ^g	Cimetidine	5 × 10 ⁻²	5.2 ± 0.7	71.1	2.5 ± 0.4	66.6	2.3 ± 0.3	46.5
	Histamine	1 × 10 ⁻³	16.2 ± 1.2	10.4	9.7 ± 0.9	-29.3	5.2 ± 0.8	-20.9
	—	—	54.1 ± 4.2	—	40.4 ± 3.4	—	28.1 ± 1.9	—
	Clemastine	2.5 × 10 ⁻⁵	26.2 ± 3.1	51.5	21.2 ± 2.2	47.5	13.2 ± 1.0	53.0
	Cimetidine	5 × 10 ⁻²	28.4 ± 2.8	47.5	23.3 ± 2.8	42.3	14.5 ± 1.2	48.4
	Histamine	1 × 10 ⁻³	49.9 ± 4.9	7.7	35.3 ± 3.4	12.6	22.8 ± 2.8	18.8

^a Drugs were added directly to the mixture of effector and target cells in a 4-hr ⁵¹Cr-release assay.

^b Effector to target cell ratio.

^c Percentage cytotoxicity and percentage inhibition were calculated as described under Materials and Methods.

^d Erythroleukemia cell line, K562, was used in the NK assay.

^e Minus sign indicates enhancement.

^f T-Lymphoblastoid cell line, CEM, was used in the NK assay.

^g B-Leukemia cell line, SB, sensitized with anti-SB antibody, was used as target in the ADCC assay. Results are the mean ± SEM of triplicate determinations for one experiment, and four separate experiments gave similar results.

TABLE 4
Effect of Histamine and Its Antagonists on NK and ADCC Activities on PBL and T Cells^a

Drug added	Concentration (M)	PBL ^b						T cells ^c			
		NK ^d		ADCC ^e		NK		ADCC		ADCC	
		Cytotoxicity (%) ^f	Inhibition (%) ^f	Cytotoxicity (%)	Inhibition (%)	Cytotoxicity (%)	Inhibition (%)	Cytotoxicity (%)	Inhibition (%)	Cytotoxicity (%)	Inhibition (%)
—	—	54.2 ± 4.5	—	45.2 ± 5.2	—	62.1 ± 6.7	—	52.3 ± 4.8	—	—	—
Clemastine	2.5 × 10 ⁻⁵	32.0 ± 3.2	40.9	14.4 ± 2.8	68.1	26.4 ± 3.1	57.4	8.9 ± 0.9	82.9	82.9	82.9
Cimetidine	5 × 10 ⁻²	14.9 ± 1.8	72.5	15.1 ± 1.8	66.5	17.8 ± 1.9	71.3	9.9 ± 1.2	81.0	81.0	81.0
Histamine	1 × 10 ⁻³	57.8 ± 4.6	-6.6 ^g	42.1 ± 4.1	6.8	57.8 ± 3.8	6.9	49.2 ± 3.6	5.9	5.9	5.9

^a Drugs were added directly to the reaction mixture of effector and target cells in a 4-hr ⁵¹Cr-released assay at a 50:1 E:T cell ratio.

^b PBL depleted of adherent cells as described under Materials and Methods.

^c T cells were separated by rosetting with neuraminidase-treated SRBC as described under Materials and Methods and contained >92% E-rosette-positive cells.

^d NK activity was measured against the human erythroleukemia cell line, K562.

^e ADCC activity was measured against the human B-lymphoblast cell line, SB, sensitized with anti-SB sera.

^f Percentage cytotoxicity and percentage inhibition were calculated as described under Materials and Methods.

^g Minus sign indicates enhancement. The results are expressed as means ± SEM of triplicate determinations for a representative experiment, and three other experiments using PBL, and four additional experiments using T cells, produced similar results.

TABLE 5
Effect of Antihistaminics on Percoll-Enriched NK Effector Cells^a

Drug	Concentration (M)	Cytotoxicity (%) ^b	Inhibition (%) ^b
None	—	28.0 ± 2.8	
Clemastine	2.5 × 10 ⁻⁵	14.0 ± 1.0	50.0
Cimetidine	5 × 10 ⁻²	6.8 ± 0.7	75.0
Histamine	1 × 10 ⁻³	29.4 ± 3.2	-5.0 ^c

^a Drugs were added directly to the mixture of effector and target cells in a 4-hr ⁵¹Cr-release assay at a 10:1 E:T ratio.

^b Percentage cytotoxicity and percentage inhibition were calculated as described under Materials and Methods. Results are expressed as means ± SEM of triplicate determinations for a representative experiment, and four separate experiments gave similar results.

^c Minus sign indicates enhancement.

These results demonstrate that histamine antagonists affect the target recognition structure of lymphocytes rather than their lytic potential.

Preculture of Lymphocytes with Histamine Suppresses NK and ADCC Activities

Figure 1 reveals the results of a time-course study of PBL precultured with different concentrations of histamine for its effect on NK and ADCC activities. PBL precultured with histamine (1 × 10⁻² to 1 × 10⁻⁴ M) for 0 to 12 hr were unaffected regarding their NK and ADCC activities. PBL precultured with histamine for 24 hr showed significant suppressive effects on their NK and ADCC functions. Maximal effect on NK (38% suppression) and ADCC (32% suppression) activities was obtained with a

TABLE 6
Effect of Histamine on Inhibition of NK and ADCC Activities by Histamine Antagonists^a

Drug added	Concentration (M)	NK ^b		ADCC ^c	
		Cytotoxicity (%) ^d	Inhibition (%) ^d	Cytotoxicity (%)	Inhibition (%)
None	—	47.8 ± 4.2	—	35.0 ± 3.3	—
Clemastine	2.5 × 10 ⁻⁵	14.2 ± 1.8	70.2	20.4 ± 2.1	41.7
Cimetidine	5.0 × 10 ⁻²	18.0 ± 1.9	62.3	13.5 ± 1.6	61.4
Histamine	1.0 × 10 ⁻³	45.0 ± 3.7	5.8	33.0 ± 2.4	5.7
Clemastine	2.5 × 10 ⁻⁵				
+ histamine	1.0 × 10 ⁻³	29.2 ± 3.1	38.9	30.2 ± 4.0	13.7
Cimetidine	5.0 × 10 ⁻²				
+ histamine	1.0 × 10 ⁻³	32.4 ± 3.0	32.2	27.6 ± 3.6	21.1

^a Drugs were added directly to the mixture of effector PBL depleted of adherent cells and target cells at a 50:1 E:T ratio in a 4-hr ⁵¹Cr-release assay. Results are the mean ± SEM of triplicate determinations for one experiment, and four separate experiments gave similar results.

^b NK activity was measured against the human erythroleukemia cell line, K562.

^c ADCC activity was measured against the human B-lymphoblastoid cell line, SB, sensitized with anti-SB sera.

^d Percentage cytotoxicity and percentage inhibition were calculated as described under Materials and Methods.

TABLE 7
Effect of Antihistaminics on Binding of Target Cells by NK Lymphocytes^a

Drug	Concentration (M)	Target binding cells (%)	Inhibition of target binding cells (%)
None	—	37 ± 3.2	—
Clemastine	2.5 × 10 ⁻⁵	19 ± 1.8	48.6
	1 × 10 ⁻⁷	40 ± 2.7	-8.1 ^b
Cimetidine	5 × 10 ⁻²	13 ± 1.0	64.8
	5 × 10 ⁻³	34 ± 2.4	8.1
Histamine	1 × 10 ⁻³	35 ± 3.6	5.4
	1 × 10 ⁻⁷	33 ± 3.0	10.8

^a Drugs were added to the mixture of lymphocytes depleted of adherent cells and K562 target cells and incubated as described under Materials and Methods. Lymphocytes bearing at least one surface-bound target cell were counted in a hemacytometer. Results are expressed as means ± SEM of three separate experiments.

^b Minus sign indicates enhancement.

1 × 10⁻³ M histamine concentration. Table 8 shows the effects of preculture of PBL with histamine on NK and ADCC activities at different E:T cell ratios. Suppression of both NK and ADCC functions of PBL precultured with histamine was evident even at higher E:T cell ratios. Studies on the effects of histamine on lymphocyte subpopulations reveal that T lymphocytes and Percoll-enriched NK effector cells, when cultured with histamine, also show suppression of their NK activity at different E:T cell ratios (Table 9).

Lymphocytes Preincubated with Histamine Suppress NK Activity of Autologous Normal Lymphocytes

In coculture experiments to measure suppression, Percoll-separated NK-enriched lymphocytes precultured with histamine were added to a mixture of autologous effector cells and prelabeled target cells at different effector to inhibitor (E:I) ratios. Results are presented in Table 10. At E:I cell ratios of 1:1 or less, lymphocytes precultured with histamine significantly suppressed the NK activity of autologous effector cells. Similar results were obtained when total peripheral blood mononuclear cells were precultured with histamine and tested for their suppressive effect on autologous effector cells in coculture experiments (data not presented).

To rule out the possibility that crowding, steric hindrance, or cell death could cause suppression of NK activity, we examined the effect of (1) normal human RBC, (2) PBL preincubated with histamine and then heat killed (56°C for 30 min), and (3) heat-killed effector cells on the NK activity of autologous effector cells. The results demonstrate that the addition of any of these cells as inhibitors to the reaction mixture at E:I cell ratios of 1:1 or less did not produce any significant suppression of NK activity by autologous effector cells (data not presented). This suggests that the suppression of NK activity by histamine-induced suppressor cells observed in the present experiments was not caused by crowding, steric hindrance, or cell death, but by functionally active viable suppressor cells.

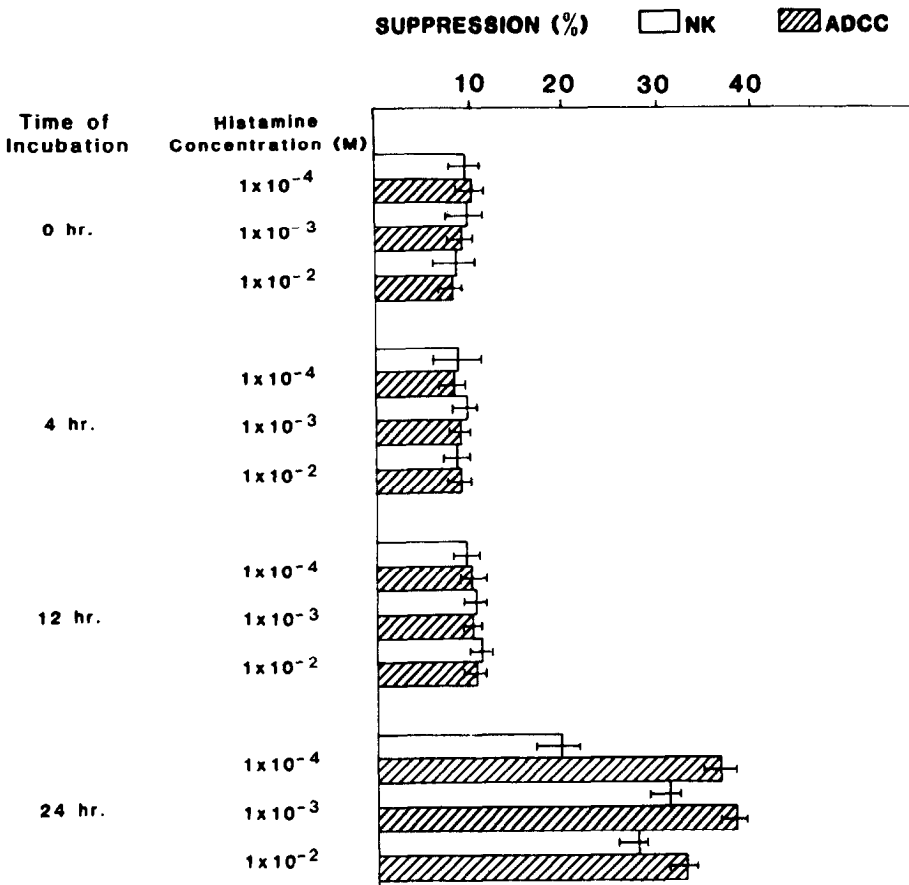


FIG. 1. Time course study of NK and ADCC activities of PBL precultured with histamine. PBL depleted of adherent cells were precultured alone or with histamine (1×10^{-2} to 1×10^{-4} M) for 0 to 24 hr, washed twice, and tested for NK and ADCC activities. Results are expressed as mean percentages of suppression of cytotoxicity \pm SEM of four separate experiments with triplicate determinations for each experiment at a 50:1 E:T cell ratio.

PBL Precultured with Histamine Show Increased Numbers of OKT8⁺ Cells

As OKT8⁺ lymphocytes are capable of suppressing various immune reactions (27, 28), experiments were undertaken to determine if lymphocytes precultured with histamine express increased number of OKT8⁺ cells. PBL depleted of adherent cells precultured with histamine (1×10^{-3} M) for 24 hr were washed and examined for OKT3⁺, OKT4⁺, and OKT8⁺ subpopulations, using monoclonal antibodies in a complement-dependent cytotoxicity assay (Table 11). PBL precultured with histamine showed no changes in either OKT3⁺ or OKT4⁺ subpopulations compared to control cultures. However, an 11% increase in OKT8⁺ cells from 21% to 32% was observed after preculture with histamine. This suggests that histamine modulates NK and ADCC activities of lymphocytes by activating OKT8⁺ suppressor cells.

DISCUSSION

NK and ADCC cells have been gaining attention because of their proposed role in defense against tumors and in the regulation of various lymphoid cell reactions

TABLE 8

NK and ADCC Activities of PBL Precultured with Histamine at Various Effector to Target Cell Ratios^a

Histamine concentration (M)	Cytotoxicity (%) ^b					
	NK ^c			ADCC ^d		
	E:T = 50:1 ^e	E:T = 20:1	E:T = 10:1	E:T = 50:1	E:T = 20:1	E:T = 10:1
0	40.0 ± 4.4	32.6 ± 3.3	20.1 ± 1.9	42.4 ± 4.4	27.9 ± 2.7	16.1 ± 1.8
1 × 10 ⁻³	28.1 ± 2.4 (29.7) ^f	19.6 ± 1.8 (39.8)	8.5 ± 0.6 (57.7)	28.0 ± 2.8 (33.9)	18.2 ± 1.6 (34.7)	4.7 ± 0.4 (70.8)

^a PBL depleted of adherent cells were cultured with or without histamine (1 × 10⁻³ M) for 24 hr, washed, and tested for NK and ADCC activities.

^b Percentage cytotoxicity was calculated as under Materials and Methods.

^c NK activity was measured against the human erythroleukemia cell line, K562.

^d ADCC activity was measured against the human B-lymphoblast cell line, SB, sensitized with anti-SB sera.

^e Effector to target cell ratio.

^f Numbers in parentheses indicate the percentage inhibition. Results are expressed as mean ± SEM of four experiments.

(29–31). Studies from our laboratory and by others have shown that NK and ADCC activities are also subject to regulation by suppressor cells or their soluble products (3, 5, 19, 20, 31–34). Earlier investigations have shown that human T cells with Fc

TABLE 9

NK Activity of T Cells and NK-Enriched Lymphocytes Precultured with Histamine^a

Histamine concentration (M)	Cytotoxicity (%) ^b					
	NK-enriched cells ^c			T cells ^d		
	E:T = 20:1 ^e	E:T = 10:1	E:T = 5:1	E:T = 50:1	E:T = 20:1	E:T = 10:1
0	73.2 ± 8.6	68.1 ± 7.9	54.6 ± 6.7	69.0 ± 5.5	62.0 ± 9.2	37.1 ± 4.5
1 × 10 ⁻⁵	77.2 ± 7.3 (-5.4) ^f	70.9 ± 6.7 (-4.1)	57.4 ± 5.3 (-5.1)	64.8 ± 4.3 (6.0)	57.2 ± 5.1 (7.7)	39.6 ± 3.9 (-6.7)
1 × 10 ⁻⁴	76.4 ± 6.6 (-4.3)	75.8 ± 7.8 (-11.3)	58.4 ± 4.8 (-6.9)	64.3 ± 6.6 (6.8)	54.7 ± 5.4 (11.7)	27.4 ± 3.2 (26.1)
1 × 10 ⁻³	60.1 ± 5.6 (17.8)	50.5 ± 4.8 (25.8)	30.3 ± 4.3 (44.5)	52.8 ± 2.8 (23.4)	23.0 ± 3.3 (62.9)	5.9 ± 3.8 (84.0)

^a T lymphocytes and Percoll-separated NK-enriched effector lymphocytes were precultured with histamine for 24 hr, washed, and tested for NK activity. Values are the means ± SEM of triplicate determinations for a representative experiment. Three separate experiments gave similar results.

^b Percentage cytotoxicity was measured against the erythroleukemia cell line, K562, as described under Materials and Methods.

^c NK-enriched lymphocytes were separated on a Percoll density gradient as described under Materials and Methods.

^d T cells were separated by rosetting as described under Materials and Methods.

^e Effector to target cell ratio.

^f Numbers in parentheses are percentage inhibition and a minus sign indicates enhancement.

TABLE 10

Suppression of Percoll-Enriched NK Cells by Autologous Lymphocytes Precultured with Histamine

Effector cells (No.) ^a	Inhibitor cells (No.) ^b	Inhibitor cell cytotoxicity (%) ^c	E:I ratio ^d	Effector cell cytotoxicity (%)		Suppression (%) ^f
				Observed ^e	Predicted ^e	
2 × 10 ⁵	—	—	—	35.2 ± 4.2	—	—
2 × 10 ⁵	1 × 10 ⁶	24.7 ± 2.4	1:5	33.2 ± 1.2	59.9	44.5
2 × 10 ⁵	8 × 10 ⁵	19.0 ± 1.2	1:4	29.3 ± 2.9	54.2	45.9
2 × 10 ⁵	6 × 10 ⁵	17.4 ± 2.6	1:3	28.7 ± 1.8	52.6	45.4
2 × 10 ⁵	4 × 10 ⁵	18.2 ± 1.5	1:2	32.8 ± 2.4	53.4	30.4
2 × 10 ⁵	2 × 10 ⁵	14.5 ± 2.8	1:1	36.4 ± 3.2	49.7	26.7

^a Percoll-separated NK-enriched lymphocytes precultured in medium alone for 24 hr were used as effectors at an E:T ratio of 10:1.

^b Autologous Percoll-enriched lymphocytes were precultured with histamine (10⁻³ M) for 24 hr, washed, and used as inhibitor cells at different ratios.

^c Percentage cytotoxicity was measured as described under Materials and Methods.

^d Effector to inhibitor cell ratio.

^e Predicted cytotoxicity equals sum of the percentage of the cytotoxicities of the effector and inhibitor cells measured separately.

^f Percentage inhibition = (predicted % cytotoxicity - observed % cytotoxicity/predicted % cytotoxicity) × 100. The results are expressed as the mean ± SEM of a representative experiment and three other experiments produced similar results.

receptors for IgG (T γ cells) are involved in NK and ADCC mechanisms (6, 10), as well as in the regulation of other immunologic reactions (8-10).

Recently histamine, a vasoactive amine, has been shown to play a role in immunoregulation (11, 13, 24, 25, 35, 36). Rocklin *et al.* (26) reported that T lymphocytes exposed to histamine *in vitro* elaborate a soluble factor which suppresses the production of migration inhibition factor and proliferation by sensitized lymphocytes. Szewczuk *et al.* (37) showed that *in vivo* injection of histamine with trinitrophenylated bovine α -globulin into mice induced the formation of autoanti-idiotypic antibodies, which regulate the immune response to trinitrophenylated bovine α -globulin. Beer *et al.* (12) recently showed that PBL precultured in the presence of histamine (10⁻⁴ to 10⁻³ M) for 24 hr could function as suppressor cells in a coculture experiment. The T γ

TABLE 11

Effect of Histamine on T-Cell Subpopulations^a

Histamine concentration (M)	OKT3 (%)	OKT4 (%)	OKT8 (%)
0	62.5 ± 3.5	22.5 ± 2.1	21.0 ± 2.8
1 × 10 ⁻³	57.3 ± 7.7	19.5 ± 0.7	32.1 ± 2.7

^a PBL depleted of adherent cells were cultured alone or with 1 × 10⁻³ M histamine for 24 hr, washed, and tested for lymphocyte subpopulations, using monoclonal antibodies by a complement-dependent cytotoxic assay. The number of positive cells stained with trypan blue were counted, using a hemacytometer. Values represent the mean ± SEM of three experiments.

subpopulation, which mediates potent NK and ADCC reactions (6, 10), has been shown to possess histamine receptors (26). It was of interest, therefore, to examine whether histamine receptor-bearing lymphocytes are involved in the regulation of NK and ADCC activities. We report here a previously unrecognized immunosuppressive effect of histamine on NK and ADCC activities of normal lymphocytes, i.e., lymphocytes precultured with histamine showed significant suppression of NK and ADCC activities, and were able to suppress the NK activity of autologous effector cells in coculture experiments. An immunoregulatory role of histamine for NK and ADCC reactions is thus proposed.

We further demonstrate that the histamine antagonists, clemastine fumarate and cimetidine, when added directly to the reaction mixture of effector and target cells, block NK and ADCC activities in a dose-dependent fashion at different E:T cell ratios. This blocking effect was evident against Percoll-separated NK-enriched effector cells and T lymphocytes. Antihistamine-induced inhibition of cytotoxicity is reversed by the addition of histamine, which at the same dose did not affect NK or ADCC activities when added directly to the reaction mixture. This is consistent with the findings of others (38–42), who demonstrated the blocking effect of NK and ADCC activities by histamine antagonists. This suggests that histamine receptors are involved in these cytotoxic mechanisms and that blocking of such receptors impedes the cytotoxic potential of lymphocytes. In TBC studies, the histamine antagonists, at NK or ADCC inhibitory doses, also reduced the number of target binding lymphocytes. This demonstrates that histamine antagonists interfere with a histamine receptor-associated target in target–effector interactions.

Suppression is operationally defined herein as the inhibition of cytotoxicity of effector lymphocytes after addition of drugs directly into the mixture of effector and target cells, or after preculturing lymphocytes with drugs. The decrease of cytotoxicity is not due to direct cytotoxicity of effector cells, since lymphocytes treated with either clemastine or cimetidine showed normal spontaneous ^{51}Cr release, and the viability was comparable with that of untreated control cultures as measured by trypan blue dye exclusion test (data not presented). Lymphocytes precultured with histamine also showed comparable viability and normal spontaneous release of ^{51}Cr with that of untreated lymphocytes (data not presented).

The natural history of effector cells involved in NK or ADCC mechanisms is still not clearly understood. In the human system, evidence indicates that at least some of the NK cells may belong to the T-lymphocyte lineage (43–45). The blocking of T-cell-mediated NK activities by histamine antagonists and the induction of suppressor cell activity by preculturing T cells with histamine may support a T-cell lineage of some NK effector cells. Our study further reveals that histamine exerts a suppressive effect on Percoll-separated NK-enriched effector cells, and histamine-induced suppressor cells were able to inhibit the NK activity of normal cells in autologous coculture experiments.

It has been shown that suppressor T cells are recognized by OKT8 monoclonal antibodies and that such T cells are known to suppress several immunologic functions (27, 28). We have examined the number of OKT8⁺ T cells present in the T-lymphocyte population precultured with histamine and have demonstrated an increase in the number of OKT8⁺ cells. The number of OKT3⁺ and OKT4⁺ subpopulations, however, remained unchanged suggesting that OKT8⁺ cells are either recruited from precursors or from OKT3⁺ or OKT4⁺ subpopulations by histamine. It is thus possible that

histamine-treated PBL may exhibit a complex phenotype with coexpression of OKT3, OKT4, and OKT8 markers. These questions are presently under investigation in our laboratory. This supports the premise that histamine activates OKT8⁺ cells to express suppressor effects on NK and ADCC reactions.

The present report demonstrates that lymphocytes with histamine receptors are involved in NK and ADCC reactions and can be activated upon culturing with histamine to express suppressor functions. This dual role of histamine receptor-bearing lymphocytes in the mediation and regulation of NK and ADCC mechanisms merits further study. Examination of any deficiencies of NK and ADCC activities in certain allergic, inflammatory or immunologically mediated diseases associated with vasoactive amines such as histamine and its antagonists, has important clinical significance.

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