Modulation of Cellular-Immune Responses in Vivo and in Vitro by Histamine Receptor-Bearing Lymphocytes

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Abstract Histamine, one of the mediators involved in the IgE-mediated reaction, was demonstrated to influence in vivo and in vitro components of cellular-immune reactions in orthochlorobenzoyl-bovine gamma globulin-immune guinea pigs. 10^{-3} M histamine reduced by half the size of a delayed hypersensitivity skin test at 24 h. Inhibition of skin reactivity by histamine could be partially reversed by H-1 receptor antagonists such as chlorpheniramine and completely prevented by H-2 receptor antagonists such as burimamide. The histamine suppression of cutaneous delayed hypersensitivity could be accounted for in part by its inhibitory effect on certain lymphocyte responses including antigen-induced migration inhibitory factor (MIF) production and proliferation. At concentrations of 10^{-3}–10^{-5} M, histamine reversibly inhibited MIF production and its action could be blocked by H-2 antagonists but not H-1 antagonists. Thus, lymphocytes bearing H-2 receptors modulate MIF production and probably lymphocyte proliferation as well. Histamine did not interfere with the macrophage response to preformed MIF. These studies indicate that immediate hypersensitivity reactions involving histamine release might influence the subsequent expression of cellular-immune reactions.

INTRODUCTION

Specific receptors for a variety of hormones have recently been detected on the membranes of a number of distinct cell types. Some of these include receptors for vasopressin, growth hormone, insulin, and histamine (1, 2). Histamine receptors modulate several functions of leukocytes such as antibody production (3), basophil histamine release (4), and lymphocyte-mediated cytotoxicity (5).

Ash and Schild demonstrated that the physiologic activities of histamine are mediated by at least two different receptors (6). The H-1 receptor mediates the contraction of intestinal and bronchial smooth muscle and the dilatation of small venules. Its effects are blocked by standard antihistamines such as mepryamine and diphenhydramine. The H-2 receptor mediates gastric acid secretion and relaxes rat uterine muscle. The latter is antagonized by a different class of antihistamines characterized by thiourea derivatives such as burimamide and metiamide (7). Plaut et al. have shown that the allogeneic lymphocyte cytotoxic reaction is regulated by cells bearing receptors of the H-2 variety (8).

In the present report, the effects of histamine were evaluated on several facets of the cellular-immune reaction in the guinea pig: the delayed hypersensitivity (DHS) [1] skin test, the antigen-induced lymphocyte proliferative response and production of macrophage migration inhibitory factor (MIF), and the response of macrophages to preformed MIF. Histamine was shown to diminish the size of a DHS skin test but not completely suppress its development. The inhibition of cutaneous DHS by histamine could be prevented by H-2 receptor antagonists such as burimamide but not by H-1 receptor blockers such as chlorpheniramine. Histamine reversibly suppressed MIF production by immune guinea pig lymphocytes and this effect was mediated by H-2 receptor-bearing cells. The lymphocyte proliferative response was only partially inhibited by histamine and the macrophage response to preformed MIF was not influenced at all.

METHODS

Drugs. Histamine hydrochloride and diphenhydramine were purchased from the Sigma Chemical Co. (St. Louis,

Dr. Rocklin is the recipient of a Research Career Development Award, AI-1K4-AI-70796.

Received for publication 3 October 1975 and in revised form 9 December 1975.

[1] *Abbreviations used in this paper:* DHS, delayed hypersensitivity; MIF, migration inhibitory Factor; OCB-BGG, orthochlorobenzoyl-bovine gamma globulin; PEC, peritoneal exudate cells.

TABLE I

Effect of Histamine on 24 h Skin Test

OCB-BGG	Histamine	Guinea pig				r^2*	Change
μg	M						%
		1	2	3	4		
50	—	6 × 5‡	7 × 6	15 × 6	10 × 10	17.6	0
50	10^{-3}	5 × 5	8 × 5	10 × 7	7 × 5	10.9	−32
		5	6	7	8		
25	—	9 × 6	8 × 4	9 × 9	10 × 9	16.0	0
25	10^{-3}	9 × 5	5 × 2	10 × 6	6 × 4	8.4	−47
		9	10	11	12		
10	—	8 × 6	10 × 8	15 × 10	10 × 8		
10	10^{-3}	5 × 4	6 × 5	5 × 4	5 × 5		
		13	14	15	16		
10	—	6 × 7	8 × 8	10 × 11	12 × 8	21.7§	0‖
10	10^{-3}	3 × 5	3 × 4	6 × 8	9 × 9	8.1§	−63‖

Suppression of cutaneous DHS by histamine. OCB-BGG-immune guinea pigs skin tested for 24 h with 10–50 μg of specific antigen with or without 10^{-3} M histamine. Lower concentrations of histamine did not affect the area of the skin test.

* Mean area of 24 h skin test ($\bar{r}^2 = [\frac{1}{2}\bar{D}]^2 \cdot \pi$).

‡ Mean induration.

§ Mean results for experiments 9–16.

‖ + Significant change ($P = 0.01$).

Mo.). Burimamide and metiamide were kindly supplied by the Smith, Kline & French Laboratories, Inc. (Hertfordshire, England). Chlorpheniramine maleate was obtained from Shering Corp. (Bloomfield, N. J.). The drugs were always freshly prepared before each experiment.

Animals. Male Hartley guinea pigs of approximately 500 g were sensitized with 100 μg of orthochlorobenzoyl-bovine gamma globulin (OCB-BGG) in saline emulsified in an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich., H37Ra). $\frac{1}{10}$ ml of the antigen-adjuvant emulsion was injected into each footpad (9).

Skin tests. OCB-BGG immune guinea pigs were skin tested 2 wk postimmunization. The flanks were prepared by electric clippers. The area was first cleansed by application of 70% alcohol. $\frac{1}{10}$ ml of antigen (10–50 μg) mixed with diluent, histamine, or histamine and antihistamine was injected intradermally. The amount of induration was measured at 24 h by an observer who was not aware of what had been injected.

Direct MIF test. Peritoneal exudate cells (PEC) from OCB-BGG immune guinea pigs were harvested according to the method of David and David (9). Exudates were induced 14–28 days after sensitization. The PEC were packed into capillary tubes and placed in Mackaness-type chambers. Tissue culture medium (TC-199) containing varying concentrations of OCB-BGG (0.01–10.0 μg/ml) with or without drugs was used to fill the chambers. The chambers were incubated at 37°C for 24 h at which time the area of migration was drawn, determined by planimetry, and the percent migration inhibition calculated by the following formula:

$$\% \text{ migration inhibition} = \left(1.0 - \left[\frac{\text{area of migration in presence of antigen}}{\text{area of migration in absence of antigen}}\right]\right) \times 100.$$

Indirect MIF test. 2–4 wk after immunization, lymph node lymphocytes were obtained from OCB-BGG immune guinea pigs using the method of David and David (9). The popliteal, axillary, and inguinal lymph nodes were teased using a mouse tooth forceps. The sediment was discarded and the cells washed twice in medium TC-199 containing 100 U of penicillin/ml and 100 μg of streptomycin/ml. Viability (60–70%) was determined by trypan blue exclusion. The final cell concentration was adjusted to 2.4×10^7 cells/ml in medium TC-199 without serum. The cell suspension was divided into two aliquots; to one aliquot, 100 μg/ml OCB-BGG was added and to the other saline was added. Varying concentrations of drugs were added along with antigen or at varying times afterward. The cell suspensions were incubated for 24 h at 37°C in a 5% CO_2-95% air atmosphere. The cell-free supernates were obtained by centrifugation and made to contain 15% guinea pig serum by volume. These supernates were assayed for MIF activity using normal guinea pig PEC in capillary tubes as described above. The percent migration inhibition was calculated using the above formula.

Lymphocyte proliferation. Lymph node lymphocytes from OCB-BGG immune animals were cultured in vitro for [^{3}H]thymidine incorporation (10). 10^7 cells/2.0 ml were cultured in TC-199 containing 15% normal guinea pig serum and 50 μg/ml OCB-BGG with or without drugs for 4 days at 37°C in a 5% CO_2-95% air atmosphere. 2 μCi/tube was added to the cultures 18 h before their termination. The DNA was extracted and the radioactivity determined by scintillation counting. The mean (cpm) of triplicate cultures were recorded and a stimulation index calculated from the ratio of cpm of antigen-stimulated cultures/cpm of unstimulated cultures. The effect of histamine and antihistamines on antigen-induced lymphocyte proliferation was compared to untreated cultures.

RESULTS

Effect of histamine on 24-h DHS skin tests. OCB-BGG-immunized guinea pigs were skin tested for the

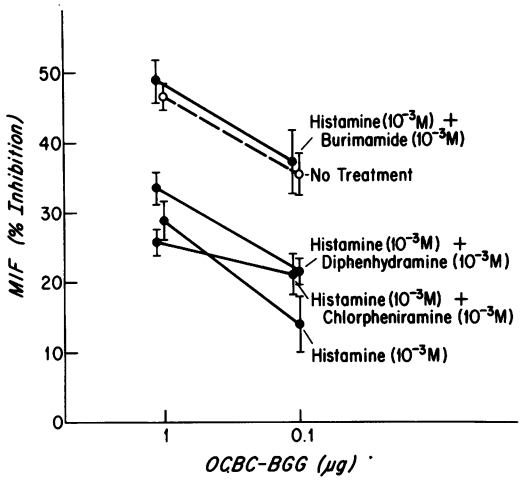

FIGURE 1 Effect of histamine on the migration inhibition of OCB-BGG-immune guinea pig PEC. In the presence of histamine (●) migration inhibition is reduced compared to control migration inhibition (○). Note H-2 receptor antagonists block the histamine effect but not H-1 receptor blockers.

development of cutaneous DHS 2–4 wk after immunization. Histamine in varying concentrations (10^{-3}–10^{-5} M) was added to the antigen preparation. In some experiments burimamide or chlorpheniramine were also added to the preparation. Table I summarizes the results of three experiments showing the effects of histamine on the expression of cutaneous DHS. At all three concentrations of antigen employed, 10^{-3} M histamine suppressed the area of the skin test (30–60%). This suppression was more pronounced at lower concentrations of antigen and was statistically significant ($P = 0.01$) at 10 μg OCB-BGG. Lower concentrations of histamine (10^{-4} and 10^{-5} M) did not significantly diminish the size of the skin reaction (data not shown). It should be noted that in some experiments, histamine did not diminish cutaneous reactivity.

The ability of H-1- and H-2-type antihistamines to block histamine suppression of the skin test is evaluated in Table II. In five experiments histamine (10^{-3} M) significantly reduced the area of the skin reaction by 43% ($P < 0.01$). Burimamide, in equimolar concentrations, completely restored the area to control values. However, chlorpheniramine (10^{-3} M) only partially corrected this suppression. Burimamide alone had no effect on the size of the skin reaction while chlorpheniramine alone reduced the size of the skin test slightly.

Effect of histamine on the direct migration inhibition assay. As a screening procedure, the effects of histamine on the direct migration inhibition system were evaluated first. PECs from OCB-BGG-immune guinea pigs were placed in capillary tubes and varying concentrations of antigen and histamine were added. Table III summarizes the results of 12 experiments in which 10^{-3} M histamine was present in the assay system for the entire incubation period. In the absence of histamine, a dose response to antigen was obtained. In the presence of 10^{-3} M histamine, the amount of migration inhibition at each antigen concentration was significantly reduced. This effect was also seen with 10^{-4} M histamine at the lowest concentration of antigen (Table IV). Indeed, the maximal effect of histamine on reducing the migration inhibition was greatest at the lower concentrations of antigen.

Since it has previously been shown that the in vitro

TABLE II

Effect of Histamine on 24 h Skin Test

Guinea pig	Skin test reagents: OCB-BGG (50 μg)	OCB-BGG +histamine (10^{-3} M)	OCB-BGG +histamine +burimamide (10^{-3} M)	OCB-BGG +histamine +chlorpheniramine (10^{-3} M)
(1) mm Induration	9.6 × 10.2	7.6 × 5.8	9.0 × 10.4	8.7 × 8.2
$\bar{r}^2$	24.5	11.22	23.52	17.87
% Change	0	−54	−4	−27
(2) mm Induration	9.0 × 11.5	6.5 × 6.5	10.3 × 9.8	8.7 × 7.2
$\bar{r}^2$	26.29	10.56	25.28	15.82
% Change	0	−60	−4	−40
(3) mm Induration	11.4 × 13.0	8.7 × 8.5	15.3 × 13.0	10.4 × 8.4
$\bar{r}^2$	37.21	18.49	50.09	22.09
% Change	0	−50	+35	−41
(4) mm Induration	10.8 × 11.0	9.0 × 8.0	11.5 × 11.4	10.0 × 8.4
$\bar{r}^2$	29.70	18.06	32.80	21.16
% Change	0	−39	+10	−29
(5) mm Induration	10.5 × 9.5	9.3 × 10.0	11.8 × 11.1	11.2 × 11.4
$\bar{r}^2$	25.0	23.3	32.80	31.92
% Change	0	−7	+30	+28
Mean				
$\bar{r}^2$	28.5±5.3	16.3±5.4	32.9±10.5	21.8±6.2
% Change	0	−43*	+15	−23

Ability of H-1 and H-2 receptor antihistamines to block histamine inhibition of skin test reactivity; OCB-BGG-immune guinea pigs injected intradermally with 50 μg of specific antigen plus histamine plus antihistamine.

* Significant change ($P < 0.01$).

TABLE III
Effect of Histamine on OCB-BGG-Immune PECs

OCBC-BGG	Migration inhibition, %		
	No treatment	Histamine (10^{-3} M)	Change
μg			
10	57±3.6*	40±3.3	−30 ($P = 0.007$)
1	49±1.8	27±2.6	−45 ($P < 0.001$)
0.1	37±2.9	15±3.4	−61 ($P < 0.001$)

Effect of histamine on the direct migration inhibition assay. PEC from OCB-BGG-immune guinea pigs with 10^{-3} M histamine present in assay system.
* Mean±SEM

effects of histamine on certain immunological responses could be blocked by H-2 receptor antagonists, but not by H-1 receptor blockers, it was of interest to see whether the same results could be obtained in the migration inhibition system. Fig. 1 summarizes the results of eight experiments in which the ability of various types of antihistamines to antagonize the histamine effect is measured. Histamine alone (closed circles) significantly reduces the migration inhibition at each antigen concentration compared to the control migration (open circles). Note that burimamide (an H-2 receptor antihistamine), in equimolar concentrations with histamine, can completely reverse the effects of histamine. Similar results were found using the drug metiamide (not shown) which is another H-2 receptor antagonist. In contrast, chlorpheniramine and diphenhydramine, H-1 receptor blockers, did not significantly rereverse the histamine effect.

Effects of histamine on the indirect migration inhibition assay. There is no way to determine from the above experiments whether histamine is operating at the level of the lymphocyte or the macrophage. Therefore, the indirect migration inhibition system was employed. The effects of histamine on the macrophage response to preformed MIF is shown in Table V. MIF and control supernates were prepared without histamine being present in the culture. 10^{-3} M histamine was then added to these supernates at the time of assay. The results in five of six experiments show that the presence of histamine in the MIF-containing supernates did not significantly interfere with the macrophage response to MIF in terms of migration inhibition.

Whether histamine suppresses MIF production by guinea pig lymphocytes was investigated next. Lymph node lymphocytes from sensitized animals were incubated with 100 μg/ml of OCB-BGG in the presence or absence of varying amounts of histamine for 24 h. The cell-free supernates were dialyzed and assayed on nonimmune PEC. Fig. 2 makes two points: histamine inhibits MIF production in a dose-response fashion and also shows the ability of certain antihistamines to reverse the effect of histamine on MIF production. Histamine significantly depressed lymphocyte MIF production and this effect occurs maximally between 10^{-3} and 10^{-5} M histamine. Moreover, burimamide can effectively return MIF production to normal, but not chlorpheniramine or diphenhydramine.

Reversibility of the effects of histamine on MIF production. Lymphocytes cultured in the absence of histamine for 24 h produce MIF whether or not they receive additional antigen for 24 h (Fig. 3g and h). In contrast, cells cultured with histamine for 48 h do not produce MIF (Fig. 3a and b). However, lymphocytes initially cultured with histamine and antigen for 24 h, washed, and recultured are able to make MIF only if fresh antigen is added (Fig. 3e and f). The addition of histamine to cultures of lymphocytes already making

TABLE IV
Effect of Histamine on OCB-BGG-Immune PECs

OCBC-BGG	Migration inhibition, %		
	No treatment	Histamine (10^{-4} M)	Change
μg			
10	70±13.4*	64±16.0	−11 (P = NS)
1	53±8.8	42±9.0	−22 (P = NS)
0.1	39±6.1	29±9.0	−25 (P = NS)
0.01	25±2.8	−1±6.0	−100 (P = 002)

Effect of histamine (10^{-4} M) on the direct MIF assay. Suppression of migration inhibition greatest at lower doses of antigen.
* Mean±SEM

TABLE V
Effect of Histamine on Macrophage Response to Preformed MIF

Expt.	Migration inhibition, %	
	No treatment	Histamine (10^{-3} M)
1	21	21
2	25	22
3	25	20
4	31	28
5	38	44
6	24	10
Mean±SEM	27.3±2.5	24.2±4.6

Indirect MIF test. Effect of histamine on the macrophage response to preformed MIF. Normal PEC in capillary tubes exposed to histamine (10^{-3} M) and MIF produced by OCB-BGG-immune lymph node lymphocytes.

MIF for 24 h does not prevent further MIF production for another 24 h (Fig. 3c and d).

The point at which histamine exerts its effect on MIF production was next determined. Lymph node cells were cultured with antigen and 10^{-4} M histamine added at varying times afterward (Fig. 4). When histamine was added at the same time as antigen or 30 min later, MIF production was significantly suppressed. However, the addition of histamine 1–2 h after antigen did not significantly reduce the amount of MIF. Thus, histamine may exert its effect at an early stage in the MIF response.

Histamine effect on lymphocyte proliferation. OCB-BGG-immune lymph node lymphocytes were assessed for their ability to incorporate increased amounts of [^{3}H]thymidine in the presence of histamine. Histamine did not significantly reduce [^{3}H]thymidine incorporation in unstimulated cultures. However, 10^{-4} M histamine significantly ($P < 0.05$) depressed [^{3}H]thymidine incorporation in antigen-stimulated cultures by an average of 42% in three experiments (Table VI). Lower concentrations of histamine were less suppressive and 10^{-3} M histamine significantly reduced radiolabel uptake in unstimulated cultures. Burimamide (not shown) effectively restores the response to control values but chlorpheniramine was unable to reverse the histamine effect.

DISCUSSION

Histamine, a mediator usually released through IgE-mediated reactions, was shown to partially diminish the expression of DHS in the skin and to profoundly alter certain lymphocyte functions in vitro. Concentrations of 10^{-3} M histamine reduced by 40–60% the size of DHS skin reactions in guinea pigs immunized with OCB-BGG in complete Freund's adjuvant. This suppression in DHS skin reactivity was completely reversed by burimamide (H-2 receptor antagonist) but only partially corrected by chlorpheniramine (H-1 receptor antagonist). It is not certain at present whether

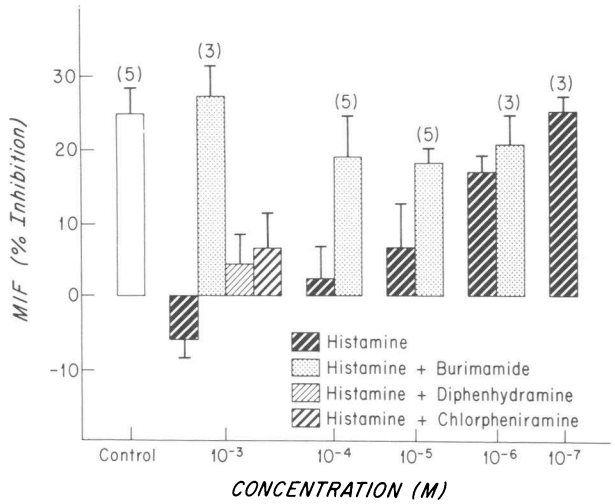

FIGURE 2 Effect of histamine on MIF production by OCB-BGG-immune guinea pig lymph node lymphocytes. Suppression of MIF by histamine is concentration related. H-2 receptor antagonists restore MIF production to normal.

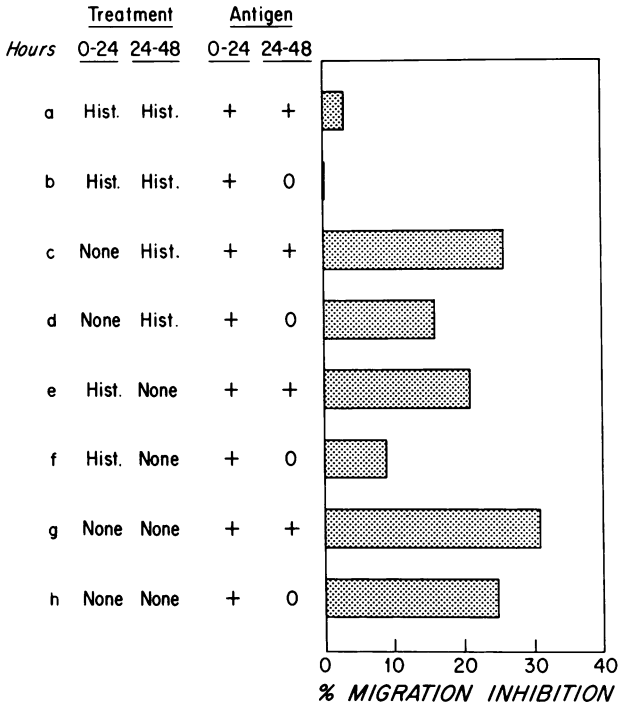

FIGURE 3 Reversibility and antigen dependence of histamine suppression of MIF production. OCB-BGG-immune cells incubated with specific antigen with or without histamine (10^{-4} M) for 24 h, washed and recultured with or without antigen, and with or without histamine for another 24 h. The second 24-h supernates were dialyzed against fresh medium and assayed for MIF activity on normal PEC.

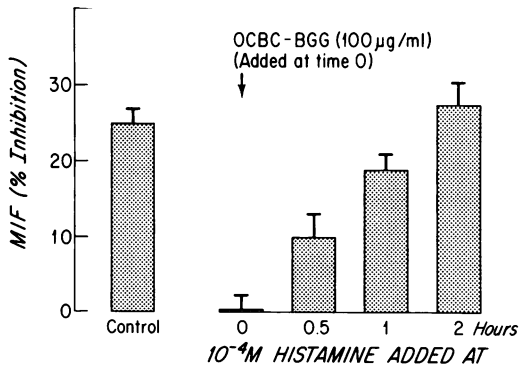

FIGURE 4 Kinetics of histamine suppression of MIF. Lymph node lymphocytes were cultured with OCB-BGG and 10^{-4} M histamine added at varying times afterward. MIF production was suppressed by the addition of histamine up to 1 h after antigen presented to cells.

TABLE VI

Effect of Histamine on [^3H] Thymidine Incorporation

OCB-BGG	Histamine	Expt. 1		Expt. 2		Expt. 3	
		cpm*	Change‡	cpm	Change	cpm	Change
μg	*M*		%		%		%
None	None	1,819	0	4,832	0	2,135	0
100	None	4,061	0	21,450	0	5,907	0
100	10^{-4}	2,308	−44§	13,717	−37§	3,282	−45§
100	10^{-5}	2,919	−29	17,518	−19	3,500	−41§
100	10^{-6}	3,039	−26	19,399	−10	3,221	−46§
100	10^{-7}	3,647	−11	17,180	−20	4,969	−16

Effect of varying concentrations of histamine on lymphocyte proliferation. OCB-BGG-immune lymph node cells activated by specific antigen and [^{3}H] thymidine incorporation measured 96 h later. Histamine in a dose-response fashion partially decreased proliferation.

* Mean cpm of triplicate cultures.

‡ Ratio of antigen and histamine to antigen alone.

§ Significant reduction ($P < 0.05$).

histamine exerts its effects on DHS skin reactions via mechanisms involving vascular permeability (i.e., "washout" of antigen from the skin site) or through its effect on lymphocyte function, or a combination of the two. The observation that chlorpheniramine (an agent which blocks histamine's effects on vascular permeability) partially restores the skin response and that burimamide (reverses histamine's effects on lymphocyte function) completely returns it to control values suggests that both mechanisms may be involved.

While it is difficult to determine how histamine influences DHS by means of skin testing, an examination of its in vitro effects on certain lymphocyte and macrophage functions related to DHS provide more information. In a dose-response fashion, histamine suppressed antigen-induced lymphocyte MIF production and proliferation. Whereas concentrations of 10^{-3} to 10^{-5} M histamine completely inhibited MIF production by OCB-BGG-immune guinea pig lymphocytes, [^{3}H]thymidine incorporation was only partially suppressed (30–40%). It is possible that histamine only partially inhibits proliferation because the kinetics of the response are much longer than those relating to MIF production. Histamine may be inactivated very early in the proliferative response and cells may then begin to undergo activation. Whereas MIF production is measured at 24 h rather than at 4 days as is proliferation. In contrast to the results obtained with skin reactions, only H-2 receptor antagonists such as burimamide and metiamide blocked the suppressive effects of histamine on lymphocyte MIF production and proliferation. Chlorpheniramine and diphenhydramine (H-1 receptor antagonists) failed to prevent histamine's action on these two in vitro cellular-immune responses. These findings indicate that lymphocytes with H-2 receptors affect MIF production and probably proliferation as well and adds yet another leukocyte function influenced by cells bearing these receptors (3–5).

Histamine reversibly inhibits MIF production; cells initially cultured with histamine for 24 h and then washed free of the drug and recultured with antigen for another 24 h can be stimulated to produce MIF (Fig. 3). Cells cultured initially with antigen but without histamine for 24 h will continue to produce MIF for another 24 h without antigen being readded to the cultures. If, however, cells are cultured with antigen and histamine for 24 h, washed, and recultured without adding further antigen for another 24 h, then MIF is not made. In contrast, cells actively producing MIF for 24 h continue to do so despite the addition of histamine to the culture. Moreover, adding histamine up to an hour after specific antigen has been initially presented to sensitized cells will significantly reduce MIF production. After this time, however, the addition of histamine does not significantly alter MIF production. These findings, taken together, indicate that histamine exerts its effects at an early step in the MIF response, perhaps interfering with an antigen-dependent step. A similar observation has recently been made by Daniels et al. regarding the production of MIF (11). They have shown that the antischistosomal drug niridazole also blocks lymphocyte MIF production at an antigen-dependent step.

Of note, histamine does not interfere with the macrophage response to preformed MIF. The latter results are consistent with those recently reported by Remold-O'Donnell and Remold who found that histamine did not stimulate adenylate cyclase activity in homogenates of guinea pig macrophages (12).

It is not clear at present how histamine exerts its

effects on MIF production or proliferation. Since lymphocyte-macrophage interaction is required for MIF production (13, 14), as well as the lymphocyte proliferative response (15–17), there are at least three points where it may act. Histamine may affect some metabolic process of the lymphocyte itself, the macrophage interaction with the lymphocyte, or a combination of the two. The inhibitory effect of histamine on the lymphocyte might be due to its ability to raise intracellular levels of cyclic AMP (2) or by activation of a suppressor or regulator cell (18, 19) which in turn could "shutoff" the MIF-producing cells or cells capable of undergoing proliferation. If histamine exerted its effects on the macrophage, this could perhaps manifest itself by an interference with lymphocyte-macrophage binding, binding of antigen to macrophages, or antigen processing by macrophages. While histamine did not significantly influence the macrophage response to preformed MIF, this would not necessarily preclude its effect at an earlier step such as that mentioned above. The question of which among these various possibilities might be related to MIF production is actively being investigated.

The observation that histamine regulates an effector function normally ascribed to cellular hypersensitivity may provide some insight into certain clinical situations. The present experiments were initiated because of a previous study which showed that lymphocytes from the majority of patients with ragweed hay fever-produced mediators (MIF and mitogenic factor) and underwent a proliferative response to ragweed antigen E despite having absent delayed cutaneous reactivity to ragweed antigen (20). Of particular interest, all of these patients had strong immediate wheal and flare reactions to ragweed which could be reduced by 50% using a standard H-1 antihistamine such as chlorpheniramine maleate. Despite the reduction in the size of the immediate reaction, no cutaneous DHS developed at the same site. It is possible that the release of histamine at the immediate hypersensitivity skin site suppressed the subsequent development of a DHS reaction. Furthermore, the results presented here suggest that the use of an H-2 receptor antagonist such as burimamide at the skin site instead of an H-1 receptor antagonist might be effective in allowing the expression of cutaneous DHS. Therefore, the investigator should be aware that IgE-mediated reactions to any antigen may interfere with the subsequent development of a DHS reaction at the same site.

Studies are presently being carried out in man to determine whether human MIF production is altered by histamine. Preliminary results suggest that antigen-induced MIF production by human lymphocytes also is suppressed by histamine using the indirect migration inhibition test. It is not clear as yet whether histamine alters the response of human monocytes to preformed human MIF.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Ms. Hollis Dolbin and Mr. Steven Flax for their excellent technical assistance.

This work was supported by U. S. Public Health Service grant AI-11729.

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