Immunoregulatory T Cells in Man

Histamine-induced Suppressor T Cells are Derived from a Leu 2+ (T8+) Subpopulation Distinct from That Which Gives Rise to Cytotoxic T Cells

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Abstract

One mechanism of histamine-mediated inhibition of the immune response in man is to activate T suppressor cells that bear the Leu 2 (OKT8) marker. The current study was undertaken to characterize the histamine-induced suppressor cell using a monoclonal antibody (9.3) shown previously to distinguish cytotoxic T cells from antigen-specific suppressor T cells. Leu 2+ cells isolated from peripheral blood were further separated with antibody 9.3 into Leu 2+, 9.3+, and Leu 2+, 9.3− subsets and each subset was incubated with different concentrations of histamine before determining their ability to suppress immune responses in vitro. The results indicate that the Leu 2+, 9.3− subpopulation includes all histamine-induced suppressor cells, that 10^{-4} M histamine is the optimal concentration for suppressor cell induction, and that exposure of Leu 2+, 9.3− cells to histamine for 30 s is sufficient to initiate the induction process. After treatment with histamine these cells inhibit both phytohemagglutinin-induced T cell proliferation and pokeweed mitogen-induced B cell differentiation. The suppression of phytohemagglutinin-induced proliferation was resistant to x-irradiation with 1,200 rad, either before or after histamine exposure, suggesting that Leu 2+, 9.3− cells need not proliferate to become suppressor cells or exert suppression. Moreover, suppression by these cells was not due to altered kinetics of the response. Finally, a histamine type 2 receptor antagonist (cimetidine) but not a type 1 receptor antagonist (mepyramine) blocked the induction of suppressor cells. On the basis of these results and our previous studies of antigen specific suppressor cells, we conclude that Leu 2+ suppressor cells in man are derived from a precursor pool that is phenotypically distinct from cells that can differentiate into cytotoxic T cells.

Introduction

Histamine can inhibit a variety of lymphocyte functions, such as the proliferative response to mitogens and alloantigens (1, 2), antibody synthesis (3), cell-mediated lympholysis (4–7), and production of lymphokines (8). Although histamine may inhibit immunity by acting directly on lymphocytes, such as antibody-secreting B cells (3), studies from several laboratories

indicate that histamine can also regulate the immune response indirectly via the induction of suppressor T cells (9–12). These histamine-induced suppressor cells have been shown to be included in the Leu-2+ (OKT8+) suppressor/cytotoxic subset (13–17) of T cells (11, 12) and not in the Leu 3+ (OKT4+) helper/inducer subset (14, 16–18).

We have shown recently that the Leu 2+ population can be divided with a murine monoclonal antibody, 9.3, into subpopulations that include precursors of antigen specific cytolytic (Leu 2+, 9.3+) and suppressor cells (Leu 2+, 9.3−) (19–21). The aim of this study was to characterize these subpopulations of Leu 2+ cells according to their ability to suppress the immune response after stimulation with histamine. The results indicate that the Leu 2+, 9.3− subpopulation includes all histamine-induced suppressor T cells, and that after treatment with histamine, Leu 2+, 9.3− cells inhibit proliferative responses of autologous Leu 3+ cells to a variety of stimuli. Moreover, the induction of suppression seems to be mediated by H_2 receptors on Leu 2+, 9.3− cells.

Methods

Drugs. Histamine dihydrochloride, cimetidine, and mepyramine maleate were purchased from Sigma Chemical Co., St. Louis, MO. Initial experiments (Fig. 1) indicated that 10^{-4} M or 10^{-3} M concentrations of histamine reproducibly induced T suppressor cells under the conditions described below, and therefore, these concentrations were used in subsequent experiments.

Monoclonal antibodies. Anti-Leu 2a and anti-Leu 3a antibodies were provided by Dr. R. L. Evans, Memorial Sloan-Kettering Cancer Center, New York, and have been described previously (13–15). Antibody 9.3 was provided by Dr. J. A. Hansen, Fred Hutchinson Cancer Research Center, Seattle, WA. Initial studies of 9.3+ and 9.3− T cells have been described (19–22).

Isolation of E-rosetting and nonrosetting lymphoid cells. Peripheral blood mononuclear cells from healthy volunteers were obtained by Ficoll-Hypaque gradient centrifugation of fresh defibrinated blood (23). T cells and non-T cells were isolated by a single step rosetting method (24) using 2-aminoethylisothiouronium bromide hydrobromide (Sigma) treated sheep erythrocytes. Rosette-forming (T) cells were separated on a second Ficoll-Hypaque gradient and freed from sheep erythrocytes by hypotonic lysis. The T cell fraction contained >95% E-rosette forming cells. The non-T cell fraction included 40–50% surface Ig positive (B) cells, 30–50% alpha naphthyl acetate esterase positive phagocytic, plastic-adherent monocytes, and 5–10% nonadherent non-phagocytic surface Ig negative, non-T, non-B cells.

Isolation of T cell subpopulations. Purified subsets of T cells were obtained by a "panning" technique that permitted the fractionation of fresh T cells into Leu 2+/Leu 2− or Leu 3+/Leu 3− subpopulations (16, 17). By using indirect immunofluorescence and cytofluorographic analysis of Leu 3 panned cells, <2% of the unbound cells and >95% of the bound cells were Leu 3+. Similar purity of subsets was achieved when anti-Leu 2 was used as the panning antibody. The Leu 3− population was further separated into 9.3+ and 9.3− subsets by panning with the 9.3 antibody. Cytofluorographic analysis of the 9.3−

subpopulation of Leu 3− cells showed that <3% of the unbound cells were 9.3+ compared with >95% of the bound cells. Isolated subsets were suspended in RPMI 1640 medium supplemented with 25 mM Hepes, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% heat-inactivated pooled human serum, hereafter referred to as complete medium.

Cell cultures. Stimulation with phytohemagglutinin (PHA)[1] (PHA-P, Burroughs Wellcome and Co., Beckingham, Kent, England) was carried out for 4 d at 37°C in an atmosphere of 5% CO_2, 95% air. To determine the effects of histamine-treated lymphocytes on the response to PHA, 1×10^5 Leu 3+ responder cells and 1.5×10^4 non-T accessory cells (irradiated to 1,500 rad) (cesium-137 unit, J. L. Shepherd and Associates, Glendale, CA) were co-cultured with different numbers (1.25×10^4 to 1×10^5) of histamine-activated cells (regulators) and 0.1 μg/ml PHA in a 0.2-ml vol. Assays were performed in triplicate and 1 μCi/well of [^{3}H]thymidine was added 18 h before harvesting with a MASH II apparatus (Microbiological Associates, Walkersville, MD). The incorporation of radiolabel in the cultures was measured by a liquid scintillation spectrometer (LS 7000; Beckman Instruments, Inc., Fullerton, CA) and the results are expressed as cpm±SEM.

A polyclonal plaque-forming assay (25) was used to detect Ig-secreting B cells after their activation with pokeweed mitogen (PWM). Briefly, histamine-activated cells or control cells were co-cultured at a ratio of 1:1 with autologous Leu 3+ cells and 1×10^5 autologous non-T cells in the presence of 1:100 PWM (Gibco Laboratories, Grand Island, NY). After 8 d of culture the cells were harvested and the number of plaque-forming cells (PFC) counted as described. The results are expressed as the mean±1 SD of the number of PFC generated by 10^6 non-T cells originally cultured. Percent suppression was calculated by the formula $1 − $ (PFC in the presence of regulators)/ (PFC in the presence of control cells) $\times$ 100.

Induction of suppressor T cells with histamine. Leu 2+, Leu 2+, 9.3−, or Leu 2+, 9.3+ cells were cultured separately at a concentration of 1×10^6 cells/ml in complete medium alone (control cells) or with histamine (10^{-3} M or 10^{-4} M) in the presence or absence of 2×10^{-5} M cimetidine (H_2 antagonist) or 10^{-6} M mepyramine (H_1 antagonist) for 12 h at 37°C. Leu 2+ subsets were also cultured with cimetidine or mepyramine alone. After incubation the cells were extensively washed and resuspended in complete medium.

Results

Suppression of PHA response by T cell subsets preincubated with histamine. Preliminary studies confirmed that incubation of Leu 2+ cells with histamine for 12 h activated cells that suppressed the response of Leu 3+ cells to PHA (Fig. 1). Similar treatment of Leu 3+ cells with histamine failed to induce suppressor cells (data not shown). To determine the optimal concentration of histamine for induction of suppressor cells, Leu 2+ cells were cultured in the presence of varying concentrations of histamine for 12 h before assays of suppressor function. As shown in Fig. 1, generation of suppression was directly related to histamine concentration and maximal generation required a concentration of 10^{-3} or 10^{-4} M. Values in Fig. 1 reflect the proliferation of both Leu 3 responder cells as well as Leu 2 regulator cells to PHA. Net suppression is determined by comparing the proliferation with and without histamine pretreatment of Leu 2+ cells at each regulator to responder ratio. In separate experiments both Leu 2 and Leu 3 cells were preincubated with varying concentrations of histamine (10^{-2}–10^{-6} M) and tested for their ability to respond
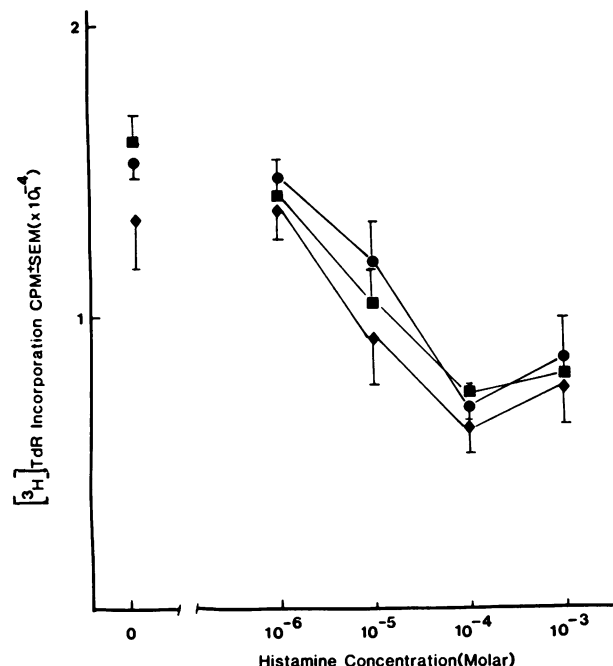

Figure 1. Effect of concentration of histamine on the generation of suppressor cells. Proliferative response to PHA of Leu 3+ cells in the presence of 1×10^5 (●), 5×10^4 (■), or 2.5×10^4 (♦) Leu 2+ cells that had been exposed to varying concentrations of histamine. Tdr, thymidine.

to PHA. The responses of both subsets were unaltered by histamine pretreatment (data not shown).

To determine if subpopulations of Leu 2+ cells differ in their ability to suppress the immune response after their exposure to histamine, Leu 2+, 9.3−, and Leu 2+, 9.3+ cells were incubated with or without 10^{-4} M histamine at 37°C for 12 h. Thereafter, the cells were washed extensively and co-cultured in graded numbers with autologous Leu 3+ responder cells, accessory non-T cells (irradiated to 1,500 rad), and PHA. Fig. 2 shows that Leu 2+, 9.3− cells preactivated with histamine suppressed the response of Leu 3+ cells to PHA. The degree of suppression depended on the number of histamine-activated Leu 2+ cells used and maximum suppression was detected at 1:1 and 2:1 ratios of responder to regulator cells. As in Fig. 1, values in Fig. 2 reflect proliferation of both responder and regulator cells to PHA. Net suppression is therefore determined in the same manner. In some experiments, untreated Leu 2+, 9.3+ cells had a slight suppressive effect on proliferation. However, pretreatment of Leu 2+, 9.3+ cells with histamine failed to induce any additional suppression, and on the contrary, often enhanced proliferation in indicator cultures.

Effect of x-irradiation on suppressor cell activation and function. In many systems, activation of suppressor cells is sensitive to doses of x-irradiation that prevent their proliferation. To determine if histamine-induced suppressor cells must proliferate in order to exert suppression, Leu 2+ 9.3− cells were irradiated with 1,200 rad before or after exposure to histamine and then tested for their ability to inhibit the response to PHA. Figs. 3 A and B show that x-irradiation of Leu 2+, 9.3− cells before or after their exposure to histamine had little or no effect on their ability to suppress the proliferative response of Leu 3+ cells to PHA.

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1. *Abbreviations used in this paper:* PFC, plaque-forming cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen; Tdr, thymidine.
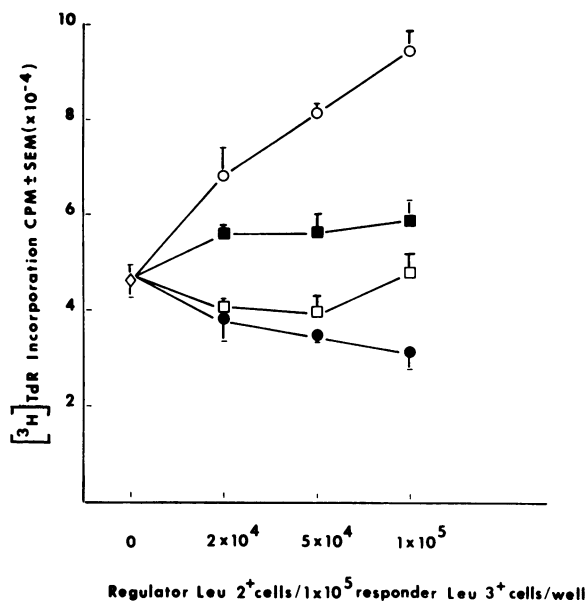
Figure 2. Proliferative response of Leu 3+ cells to PHA in the absence (◇) or presence of 10^{-4} M histamine-activated Leu 2+, 9.3— (●), or Leu 2+, 9.3+ (■) cells, or nonactivated Leu 2+, 9.3— (o), or Leu 2+, 9.3+ (□) cells.

Kinetics of suppressor cell activation. In all of the experiments described above, test cells were incubated in histamine-containing medium for 12 h before their addition to fresh autologous lymphocytes and mitogen. Additional experiments confirmed that a minimum of 3 h was required for the generation of detectable suppressor cells and generation of maximal suppression required at least 6 h incubation (Fig. 4 *A*). On the other hand, the possibility existed that histamine was not required in the medium for the entire period. To explore this possibility, Leu 2+, 9.3— cells were incubated in histamine-containing medium for shorter periods, washed, and resuspended in fresh medium lacking histamine for the remainder of the 12-h incubation before suppressor cell assay. As shown in Fig. 4 *B*, incubation of fresh Leu 2+, 9.3— cells in histamine for as little as 30 s (the shortest period tested) was sufficient to initiate the generation of suppressor cells.

Effects of H_1 and H_2 receptor antagonists on suppressor cell induction. In an attempt to determine the roles of H_1 and H_2 receptors in the histamine induction of Leu 2+, 9.3— suppressor cells, Leu 2+ subsets were cultured with 10^{-4} M histamine in the presence of either H_1 or H_2 antagonists for 12 h at 37°C, washed extensively, and then examined for their suppressor effect. As shown in Fig. 5, the presence of a saturating concentration of an H_1 antagonist, mepyramine (26), did not affect the induction of suppressor cells with histamine. However, cimetidine, an H_2 receptor antagonist, prevented the activation of suppressor cells by 10^{-4} M histamine. Even when mepyramine and cymetidine were tested at equimolar concentrations (2×10^{-5} M), only cymetidine prevented the induction of suppressor cells by histamine (data not shown). In experiments not shown, the same concentrations of mepyramine and cimetidine alone had no detectable effect on Leu 2+, 9.3—, or Leu-3+ proliferation.

Effect of histamine-activated suppressor T cells on kinetics of the PHA response. As shown in Fig. 6, the suppressive effect
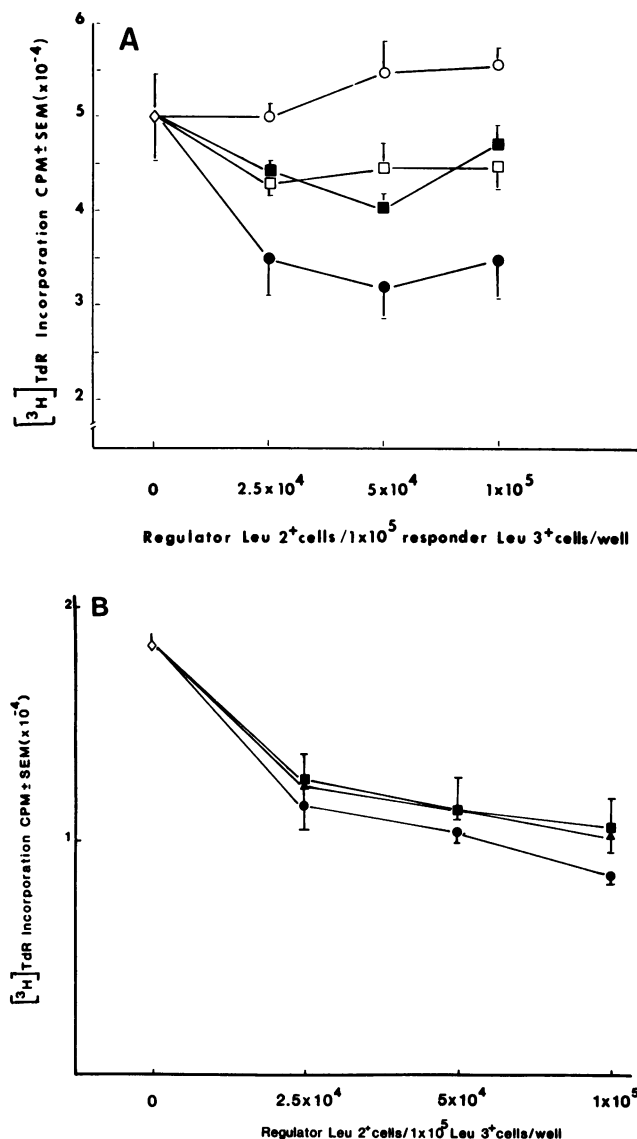

Figure 3. Effect of irradiation on suppressor cell generation and function. (*A*) Proliferative response of Leu 3+ cells to PHA in the absence (◇) or presence of 10^{-4} M histamine-activated and then irradiated (1,200 rad) Leu 2+, 9.3— (●) or Leu 2+, 9.3+ (■) cells, or nonactivated and irradiated Leu 2+, 9.3— (o), or Leu 2+, 9.3+ (□) cells. (*B*) Proliferative response of Leu 3+ cells to PHA in the absence (◇) or presence of histamine-treated Leu 2+, 9.3— cells (●), Leu 2+, 9.3— cells irradiated (1,200 rad) before histamine treatment (■), or Leu 2+, 9.3— cells histamine-treated and then irradiated (▲).

of histamine-activated Leu 2+, 9.3— cells was not due to a shift in kinetics of the proliferative response of Leu 3+ cells to PHA. Maximal suppression occurred at day 4 of culture, but significant inhibition was detected as early as 48 h after initiation of culture. As shown in the same figure, Leu 2+, 9.3+ cells exposed to histamine had little or no effect on the Leu 3+ cell response, regardless of the duration of culture.

Effect of histamine-induced suppressor cells on immunoglobulin synthesis. Leu 2+, 9.3+, and Leu 2+, 9.3— cells cultured with 10^{-3} M or 10^{-4} M histamine as described above, were mixed with autologous Leu 3+ and autologous non-T cells and stimulated with PWM. Immunoglobulin-secreting
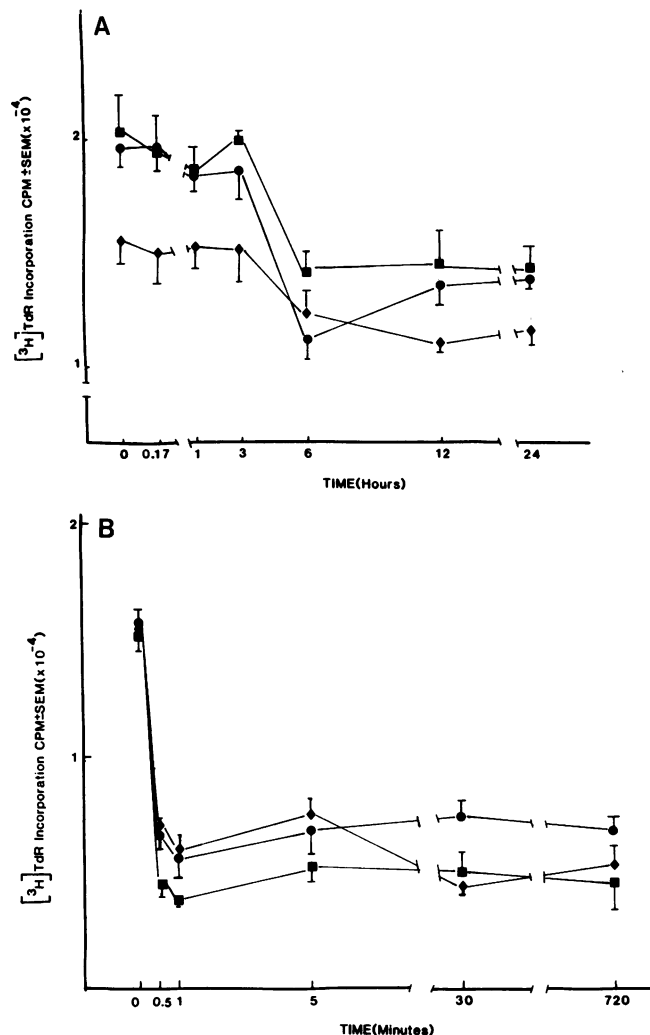
A

[3H]TdR Incorporation CPM ±SEM(x10⁻⁴)

TIME(Hours)

0 0.17 1 3 6 12 24

B

[3H]TdR Incorporation CPM±SEM(x10⁻⁴)

TIME(Minutes)

0 0.5 1 5 30 720

Figure 4. The effect of duration of histamine exposure on the generation of suppressor cells. (*A*) Leu 2+, 9.3− cells were exposed to histamine for 0–24 h, then tested at 1×10^5 (■), 5×10^4 (●), or 2.5 $\times 10^4$ (♦) cells/well for their ability to suppress the proliferative response of Leu 3+ cells to PHA. (*B*) Leu 2+, 9.3− were exposed to histamine for 0–12 h, washed, and resuspended in fresh medium for the remainder of a 12-h incubation. These cells, at 1×10^5 (■), 5×10^4 (●), or 2.5×10^4 (♦) cells/well, were then tested for their ability to suppress the proliferative response of Leu 3+ cells to PHA.

cells in these cultures were measured after 8 d with a reverse hemolytic plaque assay. The results of two representative experiments are shown in Table I and demonstrate that preincubation of Leu 2+, 9.3− cells with histamine enhanced the suppressive effect of these cells on the generation of PFC. Although there is variation in the degree of suppression between experiments, in both experiments the potency of suppression is related to the concentration of histamine present during the preincubation phase.

In another series of experiments H_1 and H_2 receptor antagonists were added with histamine to define the role of histamine type 1 and 2 receptors on suppressor cell activation. As shown in Table II, mepyramine did not significantly reduce the histamine-induced suppressive activity of Leu 2+, 9.3− cells on immunoglobulin synthesis. However, cimetidine completely blocked the effect of 10^{-4} M histamine and partially reversed the effect of 10^{-3} M histamine. Leu 2+, 9.3+ cells

[3H] TdR Incorporation CPM ±SEM(x10⁻⁴)

0 2.5x10⁴ 5x10⁴ 1x10⁵

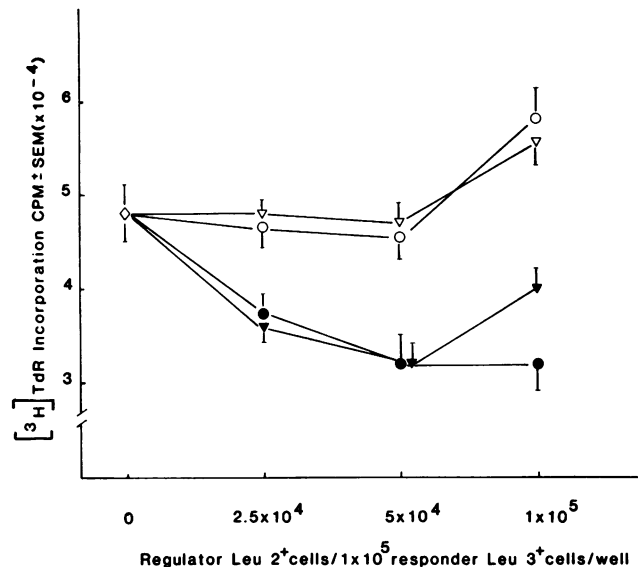Regulator Leu 2⁺cells/1x10⁵ responder Leu 3⁺cells/well

Figure 5. Effect of histamine antagonists on histamine-mediated induction of Leu 2+, 9.3− suppressor cells. PHA response of Leu 3+ cells alone (◊) or in the presence of Leu 2+, 9.3− cells precultured in medium (o), 10^{-4} M histamine (●), histamine plus 10^{-6} M mepyramine (H_1 antagonist) (▼), or histamine plus 2×10^{-5} M cimetidine (H_2 antagonist) (∇).

failed to inhibit PFC formation, and incubation of these cells with histamine in the presence or absence of H_1 or H_2 antagonists did not significantly modify the number of PFC. Incubation of Leu 2+, 9.3−, or Leu 2+, 9.3+ cells with either H_1 or H_2 antagonist alone at the concentrations used, did not significantly modify the number of PFC (data not shown).

[3H] TdR Incorporation CPM ±SEM(x10⁻⁴)

DAYS

2 4 6

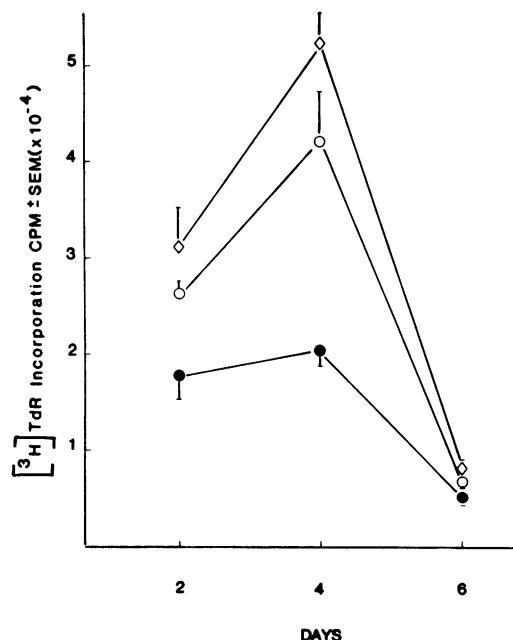*Figure 6.* Kinetics of PHA response of Leu 3+ cells alone (◊) or in the presence of an equal number of Leu 2+, 9.3+ precultured with 10^{-4} histamine (o), or Leu 2+, 9.3− cells precultured with 10^{-4} M histamine (●).

Table I. Effect of Histamine-treated Leu 2+ Subsets on PWM-Induced Immunoglobulin Synthesis

Regulators*	Experiment 1			Experiment 2		
	Treatment	PFC‡	Suppression	Treatment	PFC	Suppression
			%			%
Leu 2+, 9.3−						
	None	102,633±14,500		None	81,000±2,666	
	Histamine (10⁻⁴ M)	62,216±10,333	39	Histamine (10⁻⁴ M)	63,000±7,666	22
	Histamine (10⁻³ M)	26,833±2,950	74	Histamine (10⁻³ M)	49,833±2,433	38
Leu 2+, 9.3+						
	None	100,166±7,166		None	91,333±11,166	
	Histamine (10⁻⁴ M)	86,000±7,333	14	Histamine (10⁻⁴ M)	84,333±1,333	8
	Histamine (10⁻³ M)	100,160±9,823	0	Histamine (10⁻³ M)	80,666±5,500	12

* Each culture contained PWM, 1×10^5 non-T cells, 2×10^5 Leu 3+ cells, and 2×10^5 histamine-treated Leu 2+ subpopulation as regulators.
‡ PFC±1 SD per 10^6 non-T responder cells initially cultured.

Discussion

This study provides evidence that histamine selectively triggers suppressive activity in a subpopulation of Leu 2+ cells that lacks the 9.3 marker. The induction of suppression was dependent on both the concentration of histamine and the duration of culture of Leu 2+, 9.3− cells following their exposure to

Table II. Effect of H_1 and H_2 Antagonists on Histamine-induced Activation of Suppressor Cells of Immunoglobulin Synthesis

Regulators*	Treatment	PFC‡	Suppression
			%
Leu 2+, 9.3−	None	81,000±4,706	
	Histamine 10⁻⁴ M	63,000±7,102	22
	Histamine 10⁻⁴ M + mepyramine	52,166±8,166	36
	Histamine 10⁻⁴ M + cimetidine	82,000±16,833	−1
	Histamine 10⁻³ M	49,833±2,633	38
	Histamine 10⁻³ M + mepyramine	56,666±3,166	30
	Histamine 10⁻³ M + cimetidine	65,166±3,666	20
Leu 2+, 9.3+	None	91,333±11,166	
	Histamine 10⁻⁴ M	86,333±1,333	5
	Histamine 10⁻⁴ M + mepyramine	86,666±8,833	5
	Histamine 10⁻⁴ M + cimetidine	100,000±3,333	−9
	Histamine 10⁻³ M	80,666±5,500	12
	Histamine 10⁻³ M + mepyramine	95,333±6,166	−4
	Histamine 10⁻³ M + cimetidine	101,333±7,000	−11

* Each culture contained PWM, 1×10^5 non-T cells, 2×10^5 Leu 3+ cells, and 2×10^5 regulator Leu 2+ cells. Mepyramine and cimetidine were used at 10^{-6} and 2×10^{-5} M, respectively.
‡ PFC±1 SD per 10^6 non-T responder cells initially cultured.

histamine. After incubation in 10^{-3} M or 10^{-4} M histamine for 12 h, Leu 2+, 9.3− cells maximally suppressed the in vitro proliferative response of Leu 3+ (helper/inducer) cells to PHA. The same subset of Leu 2+ cells also suppressed PWM-induced B cell differentiation. Previously, we reported that the Leu 2+, 9.3+ subpopulation includes all or nearly all class I (HLA A, B) major histocompatibility complex alloantigen-specific cytolytic cells, whereas the Leu 2+, 9.3− subpopulation includes all precursors of class II (HLA DR) major histocompatibility complex alloantigen-specific suppressor T cells (19). In addition, we have recently shown that suppressor T cells specific for a soluble antigen, purified protein derivative of tuberculin also derived from the Leu 2+, 9.3− subpopulation (21). This subset thus contains virtually all histamine-induced suppressor cells, as well as the majority of suppressor cells activated in several additional in vitro systems.

Other investigators have shown that histamine-induced suppressor T cells can inhibit the immune response to stimuli in addition to those studied here, and although suppression in these systems has not yet been shown to be mediated by the Leu 2+, 9.3− subset, it seems likely that this is the case. If a single T cell type is inducible with histamine to become suppressive of the immune response, then it is possible that a single mechanism is responsible for suppression, regardless of the stimulus. In this regard, all of the responses known to be affected by histamine-induced suppressor cells are dependent on the activation of T helper/inducer cells which are, in turn, dependent on their interaction with accessory non-T cells. Presumably, one of these latter cell types is the primary target of suppression. Our data are compatible with either possibility.

In some experiments the addition of Leu 2+, 9.3+ cells to Leu 3+ cells resulted in modest inhibition of proliferation. This inhibitory effect was variable, but always less than that of Leu 2+, 9.3− cells, and was neither augmented by histamine nor blocked by histamine antagonists. We interpret these data as indicating that the modest inhibitory effect of Leu 2+, 9.3+ cells is mediated by a mechanism distinct from that of Leu 2+, 9.3− cells.

The precise mechanism whereby histamine-activated Leu 2+, 9.3− cells suppress the immune response was not addressed in this study. However, detailed analysis of the inhibition of the proliferative response to PHA ruled out altered kinetics of

the response as an explanation for suppression, and analogous studies of the inhibition of PWM-induced B cell differentiation by histamine-activated suppressor cells gave similar results (data not shown). Furthermore, histamine-induced suppressive activity was not abolished by irradiation of Leu 2+, 9.3− cells with 1,200 rad either before or after their exposure to histamine, suggesting that these cells need not proliferate to become suppressor cells or to exert suppression. This observation is compatible with the results of other studies indicating that histamine-induced suppressor cells mediate their effects via release of soluble factors (10, 27). It would be of interest to examine the effect of supernatants of Leu 2+, 9.3− cells after their exposure to histamine.

The apparent difference between our results and those of a previous study (1) showing that suppressive activity induced by histamine was abolished by 1,200 rad may be explained by our use of purified T cell subpopulations and longer (12 vs. 3 h) incubations with histamine. Indeed, in our hands, after exposure to histamine, a minimum of 6 h incubation was required for Leu 2+, 9.3− cells to fully mature into suppressor-effector cells. However, despite this 6-h incubation requirement, as little as 30 s exposure to histamine was sufficient to trigger this differentiation process (Fig. 4 B).

Our results do not necessarily indicate that histamine exerted an immediate effect on Leu 2+, 9.3− cells, as the possibility exists that histamine remained on the cell surface following the removal of histamine-containing medium. However, we have shown recently that exposure of Leu 2+, 9.3− cells but not Leu 2+, 9.3+ cells to histamine for 30 s results in an immediate fivefold rise in intracellular adenosine $3',5'$ monophosphate (cyclic AMP). (Khan, M., P. Sansoni, E. Engleman, and K. Melmon, manuscript submitted for publication.) Furthermore, both the induction of suppressor cells and the rise in intracellular cyclic AMP induced by histamine are blocked by histamine type 2 receptor antagonists but not by H_1 receptor antagonists. Although these two effects of histamine have not yet been shown to be directly related to one another, it is interesting to speculate that binding of histamine to H_2 receptors on the surface of Leu 2+, 9.3− cells leads rapidly to the activation of intracellular adenylate cyclase, which results in the accumulation of intracellular cyclic AMP. The rise in cyclic AMP, in turn, is postulated to initiate the subsequent steps necessary for the activation of histamine-induced suppressor cells.

We have demonstrated that histamine activates a distinct subset of Leu 2+ cells to become suppressor-effector cells in vitro. Others have suggested that the release of histamine from basophils and eosinophils can directly activate suppressor cells to modify the immune response in vivo (10). Abnormal numbers of lymphocytes that have histamine receptors are present in patients with autoimmune diseases, histiocytosis-x, allergy, and lymphoma (28–30). Furthermore, antigen-specific suppressor cells bearing histamine receptors are generated during desensitization of patients with ragweed allergy (31). In case reports, the administration of the H_2 receptor antagonist, cimetidine, was associated with acute, irreversible renal allograft rejection (32). In addition, a controlled trial of cimetidine restored defective cell-mediated immunity in patients with chronic mucocutaneous candidiasis (33). It may therefore be significant that physiologic concentrations of histamine specifically induced Leu 2+, 9.3− cells to become suppressor cells. It is possible that Leu 2+, 9.3− histamine-inducible suppressor

cells are important for normal immunoregulation and that abnormal numbers or function of these cells are responsible for the previously mentioned pathologic conditions.

Acknowledgments

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