

Pharmacologic Effects of Autacoids on Subsets of T Cells

Regulation of Expression/Function of Histamine-2 Receptors by a Subset of Suppressor Cells

Manzoor M. Khan, Paolo Sansoni, Edgar G. Engleman, and Kenneth L. Melmon

Departments of Medicine, Pharmacology and Pathology, Stanford University School of Medicine, Stanford, California 94305

Abstract

Autacoids (principally histamine, beta adrenergic catecholamines, and prostaglandins E and A) have only recently been recognized as substantive moderators of a number of immune functions. If autacoids are to be considered as potential therapeutic immunomodulators, it is necessary to understand their effects on subsets of T cells while they are and are not in contact with each other. This report demonstrates that autacoid receptors are nonrandomly distributed on phenotypically and functionally distinct subsets of human T cells. Each human T cell subset responded to both histamine and isoproterenol, but the dose response curve and maximal efficacy varied widely between the subsets. The suppressor T cells were more responsive to both histamine and isoproterenol than helper/inducer T cells (T_H) or cytotoxic T cells (T_C). We found that after mitogenic stimulation the response to histamine, but not isoproterenol, was greatly increased only in T_H ($Leu\ 3^+$) and T_C ($Leu\ 2^+$, 9.3^+) subsets, and that this effect may be regulated by suppressor T cells ($Leu\ 2^+$, 9.3^-). The dramatic rise in cAMP accumulation in response to histamine in mitogen-treated T_H and T_C was totally blocked by an H_2 antagonist (cimetidine), but not by an H_1 antagonist (mepyramine). These findings indicate interdependence of (a) immunologically uncommitted subsets in their response to selected drugs, and (b) control of basal- and autacoid-induced cAMP production, as well as (c) increased qualitative and quantitative selectivity, which is caused by mitogen. If we had performed these experiments only on unseparated cells we would not have observed the remarkable selectivity of autacoid effects on subsets of T cells.

Introduction

Autacoids, such as beta adrenergic catecholamines and histamine, are mediators and modulators of complex in vivo and in vitro models of inflammation and have lately become known as moderators of immunity (1-5). These substances are made during different phases of response to antigen and are able, directly and indirectly, to affect further responses to antigen. It is possible that the concentration of autacoids in tissue during inflammation and immune response is sufficient to allow them to modify the functions of a number of cells

Address correspondence to Dr. Melmon, Department of Medicine. Dr. Sansoni's present address is Istituto di Clinica Medica Generale, Universita degli studi di Parma, Via Gramsci 14, Parma, Italy.

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that control and express both humoral and cell-mediated immunity. Histamine and beta adrenergic catecholamines modulate the activity of immunocompetent cells via specific receptors on the cell surface, but their precise distribution on subsets of T cells and the effect that different stages of development of the immune response have on them has not been determined. After binding to specific receptors on the lymphocytes, beta adrenergic agonists and histamine activate membrane adenylate cyclase and initiate the accumulation of intracellular adenosine 3',5' monophosphate (cyclic AMP). Considerable work has described immune modulatory effects caused by cAMP (6-8), but we do not know whether all of the effects of autacoids that modulate immunity work only through this second messenger or if there are alternative or additional mechanisms of action.

This report demonstrates the distinct effects of autacoids on phenotypically and functionally distinct subsets of human T cells. We demonstrate that autacoid receptors are distributed nonrandomly on subsets of T cells and that mitogens selectively affect subsets of T cells by stimulating the cells response to the ligands, presumably via effects on receptor availability. Expression or function of H_2 receptors on $Leu\ 3^+$ (helper/inducer T cells [T_H])¹ and $Leu\ 2^+$, 9.3^+ (cytotoxic T cells [T_C]) subsets may be regulated by $Leu\ 2^+$, 9.3^- (suppressor T cells [T_S]) subsets.

Methods

Isolation of subsets of lymphocytes from human peripheral blood. Mononuclear cells from peripheral blood of healthy volunteers were obtained by ficoll-hypaque gradient centrifugation of fresh defibrinated blood. T cells and non-T cells were isolated by a single step rosetting method using aminoethyloxyethylcarbonyl bromide hydrobromide (Sigma Chemical Co., St. Louis, MO)-treated sheep erythrocytes (SRBC). Rosette-forming T cells were separated on a second ficoll-hypaque gradient and freed from the SRBC 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 plastic adherent monocytes, and 5-10% nonadherent, nonsurface Ig-negative non-T and non-B cells.

Purified subsets of T cells were obtained by a panning technique which permitted the fractionation of fresh T cells into $Leu\ 3^+$ and $Leu\ 3^-$ (2^+) subpopulations. Indirect immunofluorescence and cell sorter analysis of $Leu\ 3$ panned cells showed that <2% of the unbound cells and >95% of the bound cells were $Leu\ 3^+$. Similar subset purity was achieved when anti- $Leu\ 2$ was used as the panning antibody. The $Leu\ 2^+$ (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 $Leu\ 2^+$ cells showed that <3% of the unbound cells were 9.3^+ compared with >95% of the bound cells (9-11).

Drugs and chemicals. Histamine dihydrochloride, cimetidine, and

1. *Abbreviations used in this paper:* Con A, concanavalin A; PHA, phytohemagglutinin; SRBC, sheep erythrocytes; T_C , cytotoxic T cells; T_H , helper/inducer T cells; T_S , suppressor T cells.

mepyramine maleate were purchased from Sigma Chemical Co. and forskolin was purchased from Calbiochem-Behring Corp. (La Jolla, CA). Phytohemagglutinin (PHA)-P was purchased from Burroughs Wellcome and Co. (Beckenham, England) and concanavalin A (Con A) from Pharmacia Fine Chemicals, Inc. (Uppsala, Sweden).

Measurement of intracellular cAMP after treatment with agonists. Subsets of T cells were suspended in phosphate-buffered saline (PBS) and were incubated with 10 mM isobutyl methyl xanthine at a final concentration of 20 μ M for 10 min at 37°C. Thereafter, 2×10^5 cells were incubated with histamine (10^{-2} – 10^{-5} M) or isoproterenol (10^{-4} – 10^{-7} M) in a final volume of 250 μ l for 1 min at 37°C with continuous shaking. Immediately thereafter 1 N HCl was added to a final concentration of 10% and the tubes were put on ice. The samples were neutralized with 2 N NaOH. Acetylation of samples as well as cAMP standards was performed by addition of 10 μ l of triethylamine and acetic anhydride (2:1). Acetylation improved the sensitivity of the assay by ~50–100-fold. Radioimmunoassay was performed by the method of Brooker et al. (12).

Response to agonists after stimulation of subsets of T cells with phytohemagglutinin (PHA). Subsets of T cells were suspended to 1×10^6 /ml in RPMI 1640, 25 mM Hepes, 2 mM L-glutamine, 100 μ g/ml streptomycin, 100 U/ml penicillin (all from Gibco Laboratories, Grand Island, NY), and 10% heat inactivated pooled human serum. The cells were incubated with 1 μ g/ml PHA at 37°C in an atmosphere of 5% CO₂, 95% O₂. After 24, 48, 72, and 96 h of stimulation with PHA, the cells were centrifuged and resuspended in PBS to a final concentration of 1×10^6 cells/ml. Resting cells as well as those stimulated with PHA (2×10^5) were incubated with histamine (10^{-4} M) or isoproterenol (10^{-5} M), and intracellular accumulation of cAMP was measured as described.

Response to histamine, isoproterenol, and forskolin after Con A stimulation in subsets of T cells. After 24, 48, and 96 h of stimulation with Con A (25 μ g/ml) and monocytes, under culture conditions similar to those described for PHA, the cells were centrifuged and resuspended in PBS to a final concentration of 1×10^6 cells/ml. Resting cells as well as cells stimulated with Con A were incubated with histamine (10^{-4} M), isoproterenol (10^{-5} M), or forskolin (10^{-5} M). Intracellular accumulation of cAMP was measured as described above.

Blocking the effects of histamine by H₁ and H₂ antagonists in subsets of T cells. Subsets of T cells were incubated with an H₁ antagonist, mepyramine (10^{-6} M), and an H₂ antagonist, cimetidine (10^{-5} M), in the presence of histamine (10^{-4} M) to determine if intracellular accumulation of cAMP in resting as well as stimulated cells was mediated by H₁ or H₂ receptors. These concentrations of antagonists were chosen because they specifically saturated the H₁ and H₂ receptors (13–15).

Results

Basal intracellular cAMP levels in subsets of resting T cells. As shown in Fig. 1 A, basal cAMP levels varied between subpopulations of fresh T cells. The Leu 3⁺ subset (helper/inducer cells) had lower basal cAMP than any other subset including the mixture of Leu 2⁺ cells ($P < 0.05$, $n = 6$). When Leu 2⁺ cells were fractionated with 9.3 antibody into separate subpopulations, the basal cAMP levels among Leu 2⁺, 9.3⁺ cells (T_c) increased significantly ($P < 0.05$, $n = 6$), whereas the basal cAMP levels of the Leu 2⁺, 9.3⁻ cells (suppressor T cells [T_s]) did not differ from the mixture of Leu 2⁺, 9.3⁺ cells (T_c) with Leu 2⁺, 9.3⁻ cells (T_s). The addition of excess 9.3 antibody to unseparated Leu 2⁺ cells did not alter basal cAMP levels, which suggested that the separating antibody did not have any effect on them.

Accumulation of intracellular cAMP in subsets of T cells after their treatment with histamine or isoproterenol. Leu 3⁺, Leu 2⁺, 9.3⁺, and Leu 2⁺, 9.3⁻ cells were incubated with

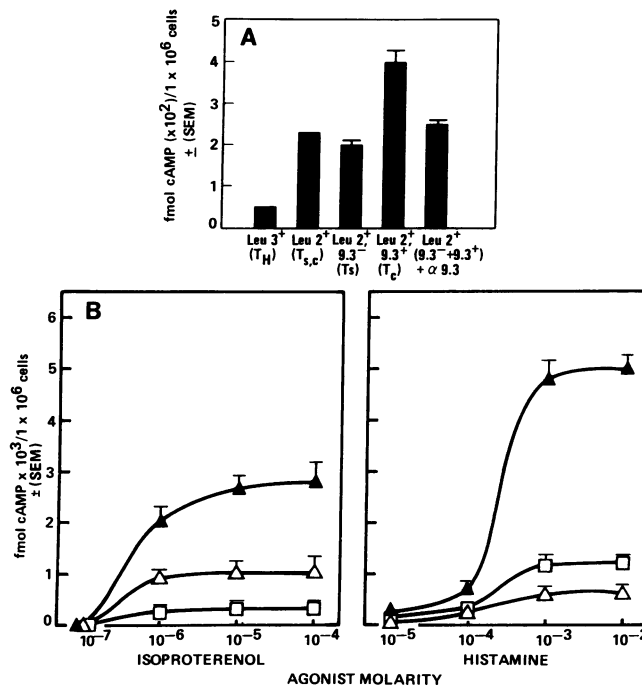


Figure 1. A shows the resting cAMP levels in subsets of T cells measured by radioimmunoassay. Basal cAMP levels are significantly different ($P < 0.05$) in Leu 3⁺ cells vs. Leu 2⁺ cells and in Leu 2⁺, 9.3⁻ cells vs. Leu 2⁺, 9.3⁺ cells. Values are means ± SEM and show one representative experiment out of six with comparable results. B shows autocoid-mediated intracellular accumulation of cAMP in Leu 3⁺ (□), Leu 2⁺, 9.3⁻ (▲), and Leu 2⁺, 9.3⁺ (△) subsets. Values are means of triplicates ± SEM in one representative experiment out of four.

histamine or isoproterenol for 1 min before the measurement of intracellular cAMP. Net accumulation of cAMP was determined by subtracting the basal levels from total stimulated intracellular cAMP (12). One representative experiment out of four is shown in Fig. 1. Each human T cell subset responded to both histamine and isoproterenol, but the dose-response curves and maximal efficacy varied widely between the subsets. The Leu 2⁺, 9.3⁻ cells were more responsive to both histamine and isoproterenol than either Leu 3⁺ or Leu 2⁺, 9.3⁺ subsets. The Leu 3⁺ subset was a significantly better responder to histamine than Leu 2⁺, 9.3⁺ cells ($P < 0.05$, $n = 4$), but the effects of isoproterenol were greater in Leu 2⁺, 9.3⁺ cells than in Leu 3⁺ cells ($P < 0.05$, $n = 4$). The simultaneous presence of the antibody which was used to separate the cells (Leu 3⁺, 9.3⁺) did not alter the agonist induced response (data not shown).

Effects of agonists on subsets of T cells stimulated with PHA. To determine if activated T cells differed from resting cells in their response to autocoids, fresh T cells and T cell subsets were each incubated with PHA (1 μ g/ml) and the activated cells were harvested after 72 h. Cells were then treated with agonists and levels of cAMP were measured. The basal cAMP levels were unchanged after the incubation with PHA (Fig. 2). The mitogen induced a slight increase in the maximal response to histamine in the mixture of T cells, but the response to isoproterenol remained unchanged. When the Leu 3⁺ cells were separated from total T cells, and treated separately with PHA, they showed a markedly enhanced

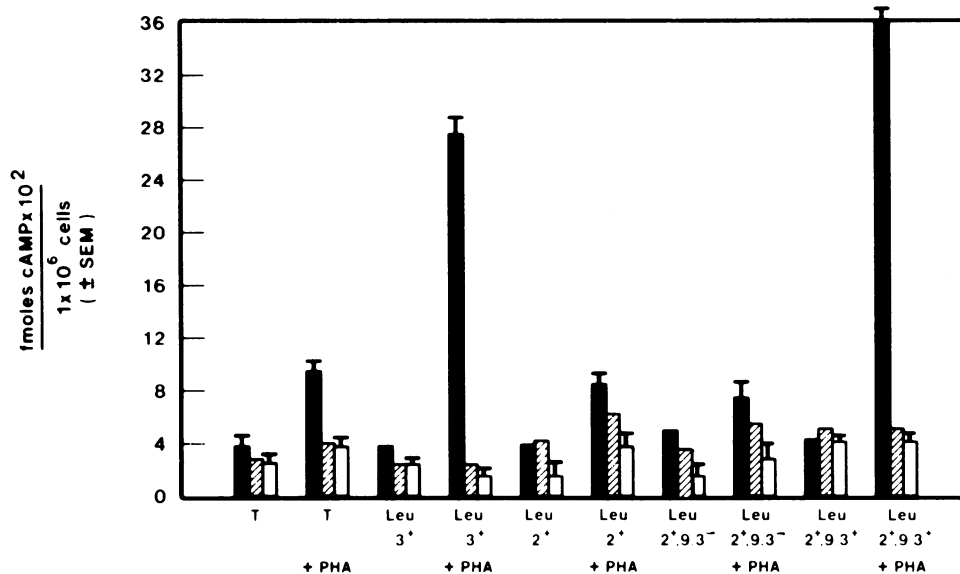


Figure 2. Intracellular accumulation of cAMP in subsets of T cells after their treatment with histamine 10^{-4} M (■) and isoproterenol 10^{-5} M (▨) is shown after stimulation of subsets of T cells with PHA (1 μ g/ml). Open bars represent basal levels of cAMP. Values are means of triplicates \pm SEM in one representative experiment out of four experiments. Levels of cAMP in Leu 3^+ and Leu 2^+ , 9.3^+ are significantly different ($P < 0.01$) in PHA treated vs. untreated cells.

response to histamine and no change in response to isoproterenol. Histamine did not excessively stimulate cAMP levels in PHA-treated Leu 2^+ cells ($T_{S,c}$) or Leu 2^+ , 9.3^- cells (T_S) above the levels seen in populations incubated in the absence of PHA. On the other hand, Leu 2^+ , 9.3^+ cells stimulated with PHA in the absence of Leu 2^+ , 9.3^- cells responded at least 15–20-fold more after stimulation with PHA than before. The change in responsiveness appeared to be agonist specific. There were no mitogen-induced changes in the response of any subset to isoproterenol (Fig. 2), whereas all subsets responded to forskolin (10^{-5} M), which demonstrated a severalfold increase in intracellular accumulation of cAMP (data not shown). T_S cells affected the T_C cells response to PHA-histamine stimulation. In addition, the mixture of T_C and T_S cells was a key factor in the response of T_H cells to PHA-histamine stimulation.

The kinetics of PHA stimulation on histamine-induced intracellular accumulation of cAMP is shown in Fig. 3. After 24 h of incubation of the subsets of T cells with PHA, only T_C cells showed increased responses to histamine, reaching a level 300% greater than before the cells were treated with PHA. After 48 h, PHA induced a 500% enhancement of the response to histamine in T_H . The enhancement was not stable in T_H cells and diminished over the next 2 d. No change in

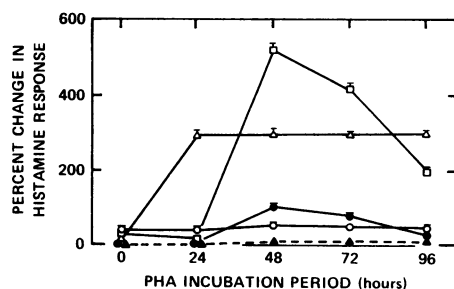


Figure 3. This figure represents the kinetics of PHA (1 μ g/ml) stimulation on histamine (10^{-4} M)-induced intracellular accumulation of cAMP in total T (●), Leu 3^+ (□), Leu 2^+ (○), Leu 2^+ , 9.3^- (▲), and Leu 2^+ , 9.3^+ (Δ) cells. One representative experiment from four experiments is shown.

response to autacoids was seen in T_S cells or in a mixture of T_S and T_C cells, which suggested that T_S cells prevent T_C cells from an augmented response to histamine. Experiments are in progress to determine the extent of this interaction. In unfractionated total T cells a slight but definite enhancement of response was observed that started after 48 h, persisted for 72 h, and returned to baseline at 96 h.

This variety of altered responses to histamine after stimulation of T cell subsets with PHA was not paralleled in response to isoproterenol (data not shown) even though the cells responded to isoproterenol before and after PHA stimulation.

Effects of agonists on subsets of T cells stimulated with Con A. The maximum responses of Con A-treated subsets of T cells to histamine and isoproterenol are shown in Fig. 4. Forskolin (10^{-5} M) was used to stimulate cellular adenylate cyclase maximally in order to determine the true maximal base upon which the autacoids could act. As shown in Fig. 4, the degree of adenylate cyclase stimulation was higher with forskolin as compared to histamine or isoproterenol. This indicated that histamine or a beta adrenergic agonist, isoproterenol, could only stimulate cAMP up to a certain limit, even though more adenylate cyclase was still available. After 48 h of incubation with Con A, forskolin-induced cyclic AMP accumulation was enhanced in all subsets. But as was the case with PHA stimulation, Con A increased histamine-induced enhanced production only in T_H and T_C cells. Once again the responses to histamine but not isoproterenol were augmented. The kinetics of the augmented response were specific to given subsets and generally paralleled the response conditioned by PHA. The basal cAMP levels in all subsets were unaffected by Con A.

Blocking the effect of histamine by H_1 and H_2 antagonists. The dramatic rise in cAMP accumulation in response to histamine in mitogen (PHA)-treated T_H and T_C cells was totally (Fig. 5) and competitively blocked by cimetidine, an H_2 antagonist. An H_1 receptor antagonist, mepyramine, did not modify the histamine-induced changes in cAMP within resting or mitogen-stimulated cells (Fig. 5). The histamine antagonists did not block forskolin-induced cAMP accumulation (data not shown) in any subset of T cells.

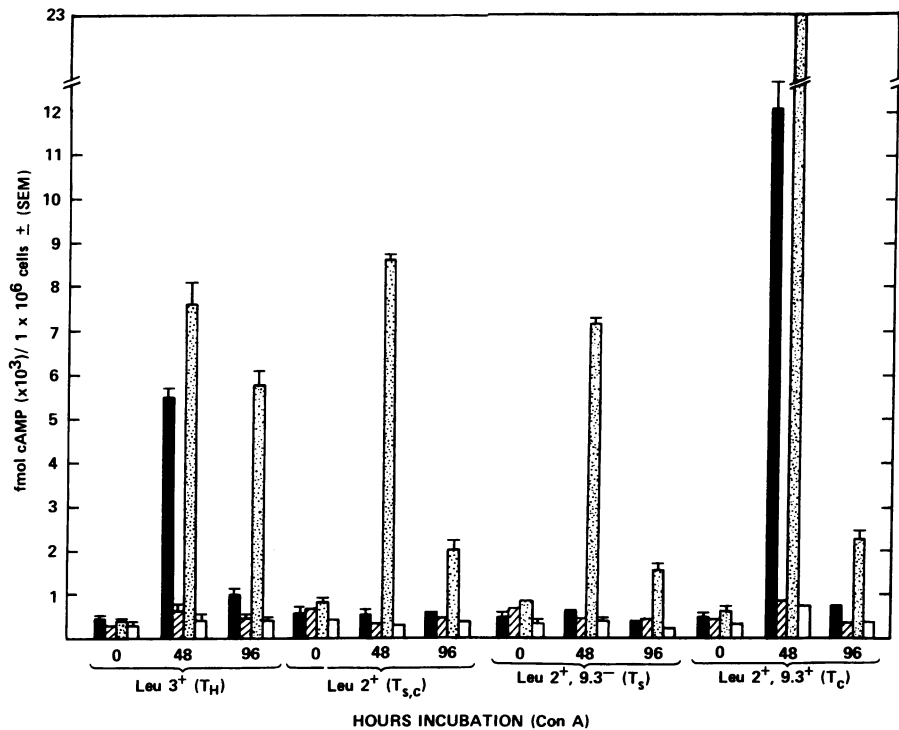


Figure 4. Effects of histamine (10^{-4} M) (▨), isoproterenol (10^{-5} M) (■), or forskolin (10^{-5} M) (▩) on intracellular accumulation of cAMP are shown after various periods of incubation of subsets of human T cells with $25 \mu\text{g/ml}$ Con A and monocytes. Open bars represent basal levels of cAMP. Values are means of triplicates. Response to forskolin is significantly different ($P < 0.01$) in all Con A-treated subsets vs. untreated cells. Response to histamine is significantly different ($P < 0.01$) only in $\text{Leu } 3^+$ and $\text{Leu } 2^+, 9.3^+$ cells in Con A-treated vs. untreated cells. One representative experiment of four experiments is shown.

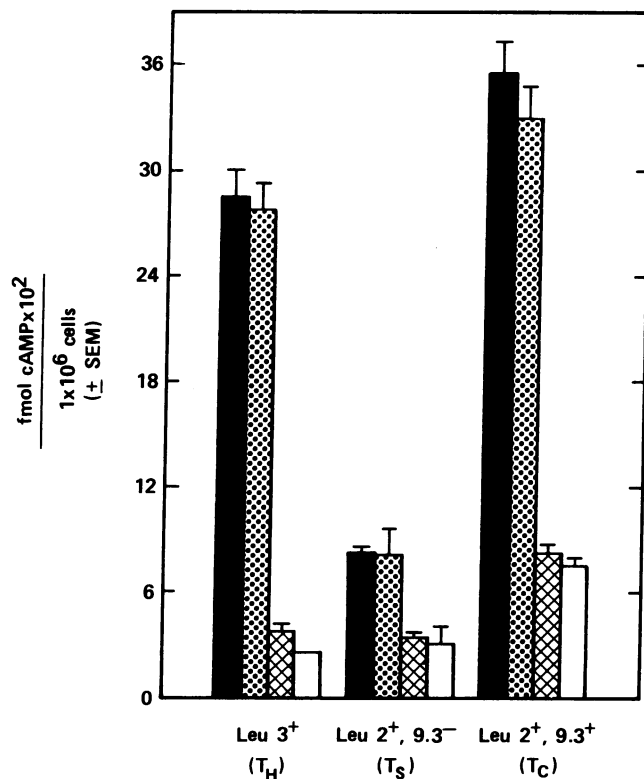


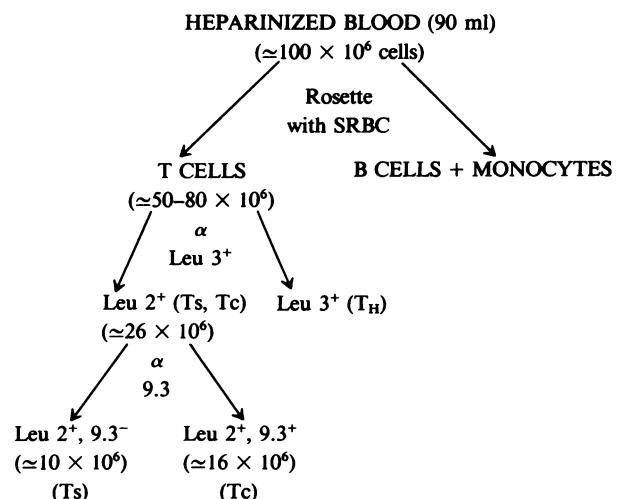
Figure 5. This figure shows the effects of an H_1 antagonist, mepyramine (10^{-6} M) (▨), and an H_2 antagonist, cimetidine (10^{-5} M) (■), on intracellular accumulation of cAMP in PHA-stimulated subsets of T cells in response to histamine (10^{-4} M) (▩). Open bars represent basal levels of cAMP. Each bar represents an average of triplicates \pm SEM in one representative experiment out of three experiments.

Discussion

The presence of receptors for autacoids on immunoregulatory T cells (T_s) was reported by Melmon et al. (16). The demonstration was largely based on the adherence of the cells to affinity columns constructed with conjugates of autacoids with albumin that were insolubilized on agarose beads. The present study evaluates the effects of autacoids on subsets of human T cells in resting as well as mitogen-stimulated states.

The $\text{Leu } 3^+$ subset was isolated from peripheral blood using Leu 3 antibody. The $\text{Leu } 2^+$ (3^-) subset was further separated using 9.3 antibody into $\text{Leu } 2^+, 9.3^+$ (T_c) and $\text{Leu } 2^+, 9.3^-$ (T_s) subsets. The $\text{Leu } 3^+$ subset includes most helper/

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inducer cells, the Leu 2⁺, 9.3⁻ subset includes most T cells capable of suppressing immunity, and the Leu 2⁺, 9.3⁺ subset includes all or nearly all (HLA, A, B) class I major histocompatibility complex alloantigen-specific T_c cells (10–12).

The pharmacologic response to histamine and isoproterenol was shared by most resting T cells. However, the rank order of potency of each of the two autacoids was specific to the given subset: Con A and PHA enhanced only the response to histamine and then only in selected subsets. The kinetics of the enhancement were also characteristic of a given subset. If we had performed these experiments only on unseparated T cells we would not have observed either the apparent control of T_s cells over the T_c cells basal cyclic AMP or the remarkable selectivity of autacoid effects.

The selectivity of qualitative and quantitative responses to histamine and isoproterenol could be explained by a nonrandom distribution and density of receptors among subsets. In similar experiments using clones of murine T cells we found distinct categories of responses to histamine and beta agonists that were peculiar to a given clone (16a). Some clones responded to both histamine and isoproterenol, others to either or neither agonist. These observations may further support the nonrandom nature of the distribution of receptors for autacoids on T cell subsets. The selectivity in the responsiveness of different populations of T cells to beta adrenergic agonists and histamine had earlier been reported (17–19). Corticosteroid resistant murine thymocytes were less responsive to isoproterenol than untreated thymocytes. The latter were more responsive than lymphoid cells from spleen, lymph node, and blood (17). Niaudet et al. (19) reported that T lymphocytes isolated from human peripheral blood produced more cAMP in response to isoproterenol than T cells isolated from human tonsils and adenoids.

An alternative explanation for the selectivity of responses could be due to differences among cells in their degree of coupling of the receptors to the adenylate cyclase pool. We have observed that different populations of T cells responded to forskolin in a selective fashion, which suggests that each subset contains a specific quantity of adenylate cyclase characteristic of that subset. In related studies we have found (16a) quantitatively additive effects of maximally effective doses of histamine and isoproterenol on cAMP accumulation in clones of mouse T_c cells. These data offer an example of histamine and beta receptors that may be linked to separate and independent adenylate cyclase pools.

Activation of T cells with mitogen (PHA or Con A) did not produce a significant change in basal levels of cAMP among the subsets. These data differ from reports (20, 21) that certain immunologic stimuli such as mitogens increased concentrations of intracellular cAMP.

Interestingly, culture of T cells with mitogens conditioned the response to one autacoid, histamine, but not to the other, isoproterenol. This conditioning occurred only in selected cells, the T_c and T_H subsets. The conditioning of responses through the T_c and T_H cells was both time and circumstance (cell mixture) dependent. Thus, the probable mechanisms of selective and organized responses to autacoids *in vivo* begin to emerge. After separation into Leu 3⁺ and Leu 2⁺ subsets, the response of Leu 3⁺ cells to histamine was augmented severalfold, whereas there was no significant change in response among the Leu 2⁺ subset. When Leu 2⁺ cells were further separated

into Leu 2⁺, 9.3⁺ and Leu 2⁺, 9.3⁻ subsets, the response to histamine among the Leu 2⁺, 9.3⁻ subset remained unaltered, whereas the response in the Leu 2⁺, 9.3⁺ subset was increased severalfold. We interpret these results as indicating that cells in the Leu 2⁺, 9.3⁻ subset may regulate the histamine response in T_H and T_c cells. Similar results were observed in the control of basal cAMP levels by T_s cells. The specificity of altered autacoid responsiveness produced by mitogens is further illustrated by its mediation only via H₂ receptors.

Forskolin stimulated the maximum possible cyclic AMP production in all mitogen-induced subsets. However, our forskolin data further suggest that mitogen stimulation does not nonspecifically increase the accumulation of cAMP caused by the two autacoids tested. The potentiation seemed unrelated to enhancement of increased availability of adenylate cyclase, because both autacoids would have been expected to potentiate the cAMP accumulation if such were the case, although only histamine did so in only selective subsets. We believe it must have worked either by increasing the number of H₂ receptors or activating some other function associated with the receptor-cyclase coupling. Thus our observations suggest that immunological stimuli have selective effects in stimulating responses to agonists. It is possible that these effects are mediated by intercellular mediators. Rocklin and others (22, 23) have reported that histamine-induced suppressor cells mediate their effects via release of soluble factors.

In related studies (13) we have found that the Leu 2⁺, 9.3⁻ subset includes all histamine-inducible suppressor cells and that after treatment with histamine these cells inhibit both PHA-induced T cell proliferation and pokeweed mitogen-induced B cell differentiation. A histamine H₂ receptor antagonist (cimetidine), but not an H₁ receptor antagonist (mepyramine), blocked the induction of suppressor cells, which suggests that activation of Leu 2⁺, 9.3⁻ cells with histamine may be mediated by H₂ but not by H₁ receptors.

To our knowledge, regulation of histamine receptor-mediated cAMP production in cytolytic T cells by T_s cells has not been previously reported. Plaut et al. (24) suggested that lymphocytes mediating cellular immunity develop receptors for histamine as a function of time after immunization. They also found an increase in the inhibitory effects on the function of cytolytic cell action correlated with the enhanced responsiveness in production of cyclic AMP. 10 d after immunization, histamine caused only ~10% inhibition of cytolysis, which gradually rose to >50% by day 16. In contrast, dibutaryl cAMP, cholera enterotoxin, isoproterenol, and prostaglandin E caused consistent inhibition of cytolytic activity regardless of time elapsed after immunization. These experiments suggest that the cytolytic mechanism itself is inhibited by cyclic AMP at all times, but that histamine receptors develop gradually on killer lymphocytes only after immunization.

We know that agents that elevate intracellular cAMP also alter the immunological activity of cells (6–8). The mechanisms by which cAMP affects the immune process are not clear. We believe that further understanding of the mechanisms by which the Leu 2⁺, 9.3⁻ subset modulates the expression or function of the H₂ receptor will be helpful in understanding the role of the autacoids and the second messenger in immune processes.

The differential ability of the cells to respond allows a structural and dissectible sequence of predominant actions caused by a ubiquitous hormone. Understanding that sequence

seems important if we are ever to use the autacoid prospectively to modulate immunity.

Acknowledgments

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