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HORMONE RECEPTORS ON CLONED T LYMPHOCYTES

Increased Responsiveness to Histamine, Prostaglandins, and β-Adrenergic Agents as a Late Stage Event in T Cell Activation[1]

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Lymphocytes have surface receptors for a variety of hormones that play an important part in modulating the immune response. Most previous studies, however, have examined the effects of hormone agonists on heterogeneous bulk populations of cells, making it difficult to precisely identify the responding target cells. We have therefore studied a set of well characterized T cell clones for a series of adenylate cyclase-linked hormone receptors and examined changes in receptor expression that occur after cell activation. All clones tested had receptors for histamine, isoproterenol, and PGE_1, but not for several other cAMP-active hormone agonists. The apparent receptor affinities and their specificities were characteristic of typical histamine H_2, β_2-adrenergic, and PGE receptors. The cAMP response to PG was higher and longer lasting than that to histamine or isoproterenol, both of which appear to undergo receptor desensitization. After activation of quiescent cells in IL-2-containing media, the cAMP response to all three ligands increased, peaking 4 to 5 days after stimulation, and then returned to basal levels as the cells ceased proliferating. Inasmuch as this effect did not require Ag, it appears that the coordinate regulation of responsiveness to these ligands is a direct result of lymphocyte activation. This increase in hormone receptor activity is functionally analogous to the up-regulation of receptors for other ligands that occurs after lymphocyte activation and further demonstrates the important immunoregulatory role played by the changing repertoire of surface receptors that is associated with activation.

Immune responses in vivo are controlled by the complex interactions between various subsets of lymphocytes, as well as influences from other, nonlymphoid cells. Many classical hormones and lymphokines have important roles in regulating immunity. Thus, lymphocytes have surface receptors for a variety of peptide hormones, including insulin and vasoactive intestinal peptide, for vasoactive amines, such as HA[2] and epinephrine, and for other low m.w. molecules, including PG (1–3). Binding of HA, PG, and β-adrenergic agents to their specific receptors stimulates membrane-bound adenylate cyclase, thereby causing an increase in the intracellular concentration of cAMP. The physiologic effects of these adenylate cyclase-activating hormones on lymphocyte function is most often one of immunosuppression. HA and PGE_1 have been shown to inhibit the proliferative responses of T lymphocytes to mitogen and to Ag and to inhibit the secretion of lymphokines (4–7). Both of these hormones also inhibit the expression of killing activity by murine Tc cells (8, 9).

In spite of the large amount of information regarding the physiologic role of hormones in immunity, the precise mechanisms by which they exert their effect have often been obscure due to the heterogeneous nature of the cell populations previously studied. Thus, although HA can increase the concentration of cAMP and inhibit the generation of cytolytic activity in immune spleen cells, it is not clear whether HA directly causes these changes in Tc cells or whether it acts via some intermediate cell type. We have therefore studied the interaction of several adenylate cyclase-activating hormones with cloned T lymphocyte lines with well characterized activities and antigenic specificities. With the use of such cells, it was previously demonstrated that murine Tc cell clones have HA-R of the H_2 subtype that are linked to adenylate cyclase and that these receptors desensitize after incubation with specific ligands (10). In the present report, we demonstrate that Tc cell clones also have specific adenylate cyclase-linked receptors for PG and β-adrenergic agents. Interestingly, the responsiveness to these hormones increases in parallel with activation of the cells, with maximal responsiveness occurring several days after stimulation by IL-2-containing media. Thus, Tc cells are subject to direct regulatory effects of HA, PG, and epinephrine, and their susceptibility to such control varies with the stage of growth after activation. This increase in hormone responsiveness suggests an important regulatory role for the changing repertoire of functional hormone receptors during T cell activation.

MATERIALS AND METHODS

Reagents. HA dihydrochloride, Iso, epinephrine, norepinephrine, phenylephrine, propranolol, con A, IBMX, and 2′-*O* monosucci-

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[2] Abbreviations used in this paper: HA, histamine; IBMX, isobutylmethylxanthine; Iso, isoproterenol; C subunit, adenylate cyclase catalytic moiety.

nyladenosine 3′: 5′-cyclic monophosphate tyrosyl methyl ester were purchased from Sigma Chemical Co. (St. Louis, MO). PGE_1, PGE_2, $PGF_{2\alpha}$, and PGI_2 were donated by Dr. J. Willis (Syntex, Palo Alto, CA). Dimaprit, impromidine, and cimetidine were kindly provided by Dr. C. R. Ganellin (Smith Kline & French Laboratories, Welwyn, Hertfordshire, U.K.). Highly specific antibodies to cAMP were obtained from Dr.G. Brooker (University of Virginia, Charlottesville, VA). Iodinated tyrosylsuccinyl-cAMP used in the RIA was prepared by the chloramine T method (11).

T cell clones. 3A1, C4.2, and B12.5 are Tc cell clones derived from C57BL/6 mice and are specific for H-d^d alloantigens (12). Clone AR-1, from a C57L mouse, also reacts with H-2^d alloantigens. 1E4 is an H-2^b-restricted cytotoxic clone against Abelson virus-induced lymphomas (13). All of these cell lines are Thy-1^+, Lyt-1^-2^+ and are passed weekly with appropriate irradiated stimulator cells, rat spleen con A supernatant as a source of IL-2, and 20 mM α-methyl-mannoside to block binding of residual con A. All of these cytolytic clones can be passed for one growth cycle (1 wk) in medium containing IL-2 (either purified or as con A supernatant) without Ag. (Further passage beyond this week requires the inclusion of antigenic stimulator cells to reinduce IL-2R needed for proliferation.) Thus, in some experiments, cells were passed in fresh medium with con A supernatant alone to resolve the effects of antigenic stimulation from those of soluble growth factor alone. Clone TSK 3 F1.1 is a keyhole limpet hemocyanin-specific helper clone of (C57BL/6 × C3H)F_1 origin, obtained as previously described (14).

Short term Ag-nonspecific clones were generated from con A-activated spleen T lymphocytes as follows: C57BL/6 spleen cells were stained with biotinylated anti-Thy-1.2 (mAb 30-H12) and FITC-avidin and were sterile sorted with a modified FACS II (Becton Dickinson, Sunnyvale, CA). One Thy-1.2^+ cell was deposited into each well of a flat-bottomed microtiter plate containing 1×10^6 irradiated (1400 rad) syngeneic spleen cells, 2.5 μg/ml con A, and 20% rat con A supernatant. Five of the resulting clones were subsequently expanded in 25-cm^2 flasks with con A and IL-2 and then assayed for hormonal responsiveness.

Stimulation of cells and assay of cAMP levels. Cells were harvested and stimulated according to previously described methods (10). Briefly, cells taken 4 or 5 days after activation were centrifuged, washed, and resuspended at a concentration of 2×10^5 to 4×10^5 cells/ml in 0.20 mM IBMX in PBS, pH 7.5. The cells were then incubated with the various drugs for 2 min at 37°C, followed by the addition of 1.0 M HCl to stop the reaction. These cell lysates were then assayed for their cAMP levels by a modification (10) of the RIA method of Brooker et al. (15). In brief, the samples were neutralized with NaOH and acetylated, and aliquots were added to 10,000 cpm ^{125}I-tyrosylsuccinyl-cAMP and an appropriate dilution of anti-cAMP antibody. Throughout the cell isolation and stimulation procedures, care was taken to avoid temperature shock, which can cause artifactual increases in cAMP levels (16). Results, unless otherwise indicated, are expressed as the mean of triplicate determinations. The SD of replicates were generally 3 to 10% of the mean.

[^{3}H]TdR incorporation. Cellular proliferation was assessed daily throughout the 1-wk growth cycle by a [^{3}H]TdR incorporation assay. Aliquots of 100 μl of cell cultures were added to flat-bottomed microtiter wells containing 120 μl of fresh medium and 1 μCi of [^{3}H]TdR (New England Nuclear, Boston, MA). After incubating at 37°C for 4 h, the samples were deposited onto glass fiber filters with an automatic multiple well harvester and then counted in a scintillation counter. All determinations were performed in triplicate.

RESULTS

Hormone responsiveness of T cell clones. A set of Tc cell clones were tested for their ability to respond to a variety of hormones with increases in their intracellular concentration of cAMP. As shown in Table I, these clones responded to HA, Iso and PGE_1. HA and Iso, a β-adrenergic agonist, stimulated comparable amounts of cAMP accumulation, generally reaching levels four- to eight-fold above the basal concentration. An Ag-specific Th cell clone also showed HA reactivity. PG elicited a much greater rise in cAMP levels than HA or Iso, with increases generally of 25- to 100-fold. Several other hormones, including serotonin, dopamine, glutamate, and D-Ala-D-Leu-enkephalin, did not elicit significant changes in cAMP levels (stimulated levels were less than or equal to 1.6 times that of basal). In addition, membrane-binding assays with [^{3}H]etorphine showed no detectable opioid receptors on these cell lines (data not shown).

TABLE I
cAMP responses of T cell clones

Cells	cAMP (pmol/10^6 cells)[a]			
	Basal	HA	Iso	PGE_1
Cytolytic clones				
C4.2	1.8	10.9	16.0	100.2
B12.5	0.7	1.7	7.2	–[b]
AR-1	1.0	3.2	4.5	41.3
1E4	1.9	6.8	4.8	56.7
3A1	1.4	7.0	–	–
Helper clone TSK 3 F1.1	0.6	3.0	–	–

[a] Basal and stimulated levels of cAMP reached 2 min after the addition of optimal concentrations of HA (100 μm), Iso (10 μm), and PGE_1 (10 μm).
[b] – = not done.

To test the generality of hormone responsiveness in cloned T cells, unselected Ag-nonspecific short term T cell clones were generated and then tested for hormonal responses. Five such clones were tested for their responses to HA, Iso, and PGE_1. All of these clones reacted to all three hormones with responses similar to those found for the Ag-specific Tc clones. Thus, including the Ag-specific clones in Table I, 11 of 11 clones tested responded to HA, 9 of 9 responded to Iso, and 8 of 8 responded to PGE_1.

Pharmacologic characterization of responses. Figure 1 shows representative concentration-response curves for HA, Iso, and PGE_1. The half-maximal response concentrations (EC_{50}) for these agents, an estimate of the apparent receptor affinity (K_a), were 1.7×10^{-5} M, 9.5×10^{-8} M, and 9.0×10^{-7} M for HA, Iso, and PGE_1, respectively. These plots are representative of many replicate experiments for the five Tc clones. As summarized in Table II, the HA H_2 agonists impromidine and dimaprit stimulated adenylate cyclase with a rank order of potencies characteristic of H_2-R whereas H_1 agonists, such as 2-methylhistamine, had no effect (see also Reference 10). The H_2 antagonists cimetidine and tiotidine blocked the cAMP rise induced by HA further indicating that the HA-R are of the H_2 type. Similarly, β-adrenergic agonists stimulated cAMP responses, and these were blocked by the β-adrenergic antagonist propranolol. The rank order of potencies (Iso > epinephrine > norepinephrine > phenylephrine) is characteristic of β_2-adrenergic receptors (17). The PG agonists PGE_1, PGE_2, and $PGF_{2\alpha}$ increased cAMP levels, whereas PGI_2 had little effect. Thus, the ligands HA, Iso, and PGE_1 interact in a specific manner with defined HA H_2, β_2-adrenergic, and PGE receptors, respectively.

Time courses of cAMP responses. Since the rapidity and duration of cAMP responses may be important in determining the physiologic signal to the cell, we examined the kinetics of intracellular cAMP accumulation after the addition of optimal concentrations of hormones. As shown in Figure 2, HA and Iso induced rapid increases in the concentration of cAMP, peaking within 5 min of stimulation. After this initial rise, both HA- and Iso-stimulated cAMP concentrations fell rapidly toward baseline, even in the continued presence of ligand. After stimulation with PGE_1, the intracellular cAMP concentration also rose quickly, plateauing at a high level 15 to 20 min after ligand addition. Unlike HA or Iso, however,

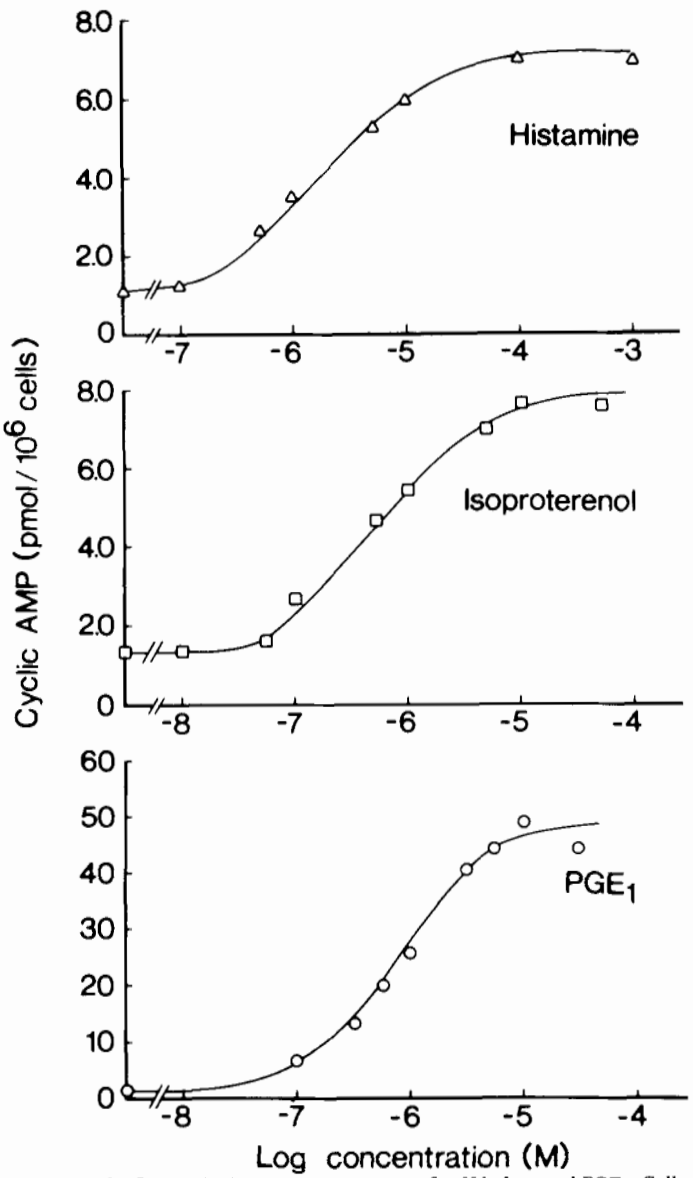

Figure 1. Concentration-response curves for HA, Iso, and PGE_1. Cells of clone C4.2 were incubated in PBS containing 0.2 mM IBMX and were then treated with the indicated concentrations of hormones for 2 min. cAMP levels were then determined as described in *Materials and Methods*. These curves are representative of many replicate experiments with C4.2 and the other four cytolytic clones.

TABLE II

Specificity of cAMP responses in Tc cell clones

Receptor Type	Agonist	EC_{50} (M)	Antagonist	K_i (M)
HA H_2	HA	1.7×10^{-5}	Cimetidine	1.4×10^{-6}
	Impromidine	1.4×10^{-5}	Tiotidine	4.0×10^{-8}
	Dimaprit	3.8×10^{-5}		
	2-Methylhistamine	2.8×10^{-3}		
β_2-Adrenergic	Iso	9.5×10^{-8}	Propranolol	1.0×10^{-9}
	Epinephrine	8.5×10^{-7}		
	Norepinephrine	4.5×10^{-5}		
	Phenylephrine	$>10^{-4}$		
PGE	PGE_1	9×10^{-7}		
	PGE_2	1.3×10^{-6}		
	$PGF_{2\alpha}$	1.0×10^{-5}		
	PGI_2	$>10^{-4}$		

PGE_1-stimulated cAMP levels remain elevated for a long period of time, undergoing little or no decrease for at least 1 h.

Modulation of hormonal responsiveness after lymphocyte activation. The expression of many lymphocyte surface receptors and Ag vary with the stage of cell activation, reflecting the changing physiologic requirements of such cells. The responses of Tc cell clones to HA, PG, and Iso were therefore measured at various time intervals after restimulation of resting cells. Cells were restimulated with Ag and con A supernatant on day 0. On subsequent days, aliquots of cells were removed and incubated with HA, and the concentrations of cAMP were determined. As shown in Figure 3, the ability of cytolytic clones to respond to HA increases after activation, peaks around day 5, and then returns to basal levels by the end of the 1-wk growth cycle. This variation in cellular responsiveness to HA parallels the rise and fall in proliferation, as assessed by [^{3}H]TdR incorporation (Fig. 3). In several experiments, we found that cells activated in IL-2-containing media alone, without allogeneic stimulator cells, responded at least as well as cells activated by both IL-2 and alloantigen. The following experiments were

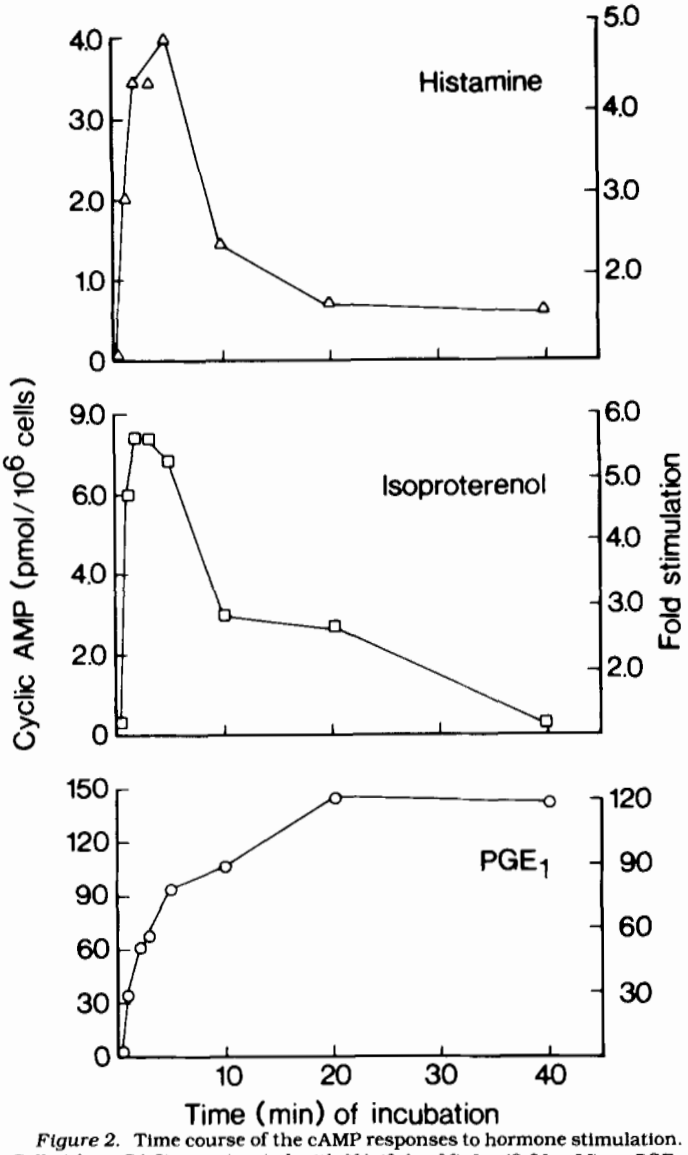

Figure 2. Time course of the cAMP responses to hormone stimulation. Cells (clone C4.2) were treated with HA (0.1 mM), Iso (0.01 mM), or PGE_1 (0.01 mM) for the indicated periods of time in the presence of 0.2 mM IBMX, and the resulting levels of cAMP were then assessed. The levels of cAMP represent stimulated levels above the basal concentration of cAMP (1.0 pmol/10^6 cells). Each point represents the mean of triplicate determinations.

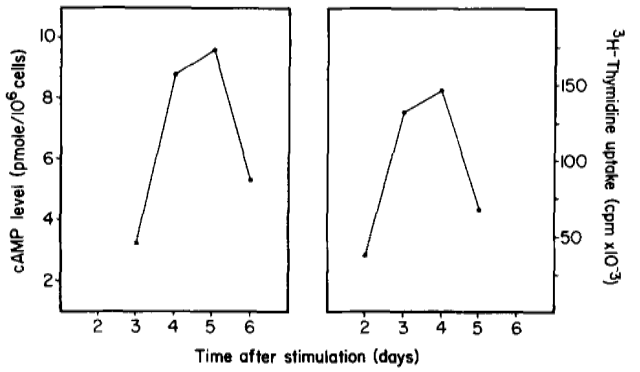

Figure 3. Variation in the expression of hormonal responsiveness as a function of time in the cell activation/growth cycle. C4.2 cells were passed on day 0 with allogeneic stimulator cells and con A supernatant. On subsequent days, aliquots of cells were removed, stimulated with 1 mM HA for 2 min, and the resulting increase in the intracellular concentration of cAMP was determined (*left*). The degree of cellular proliferation was assessed in triplicate aliquots of cells by [^{3}H]TdR incorporation as described in *Materials and Methods* (*right*).

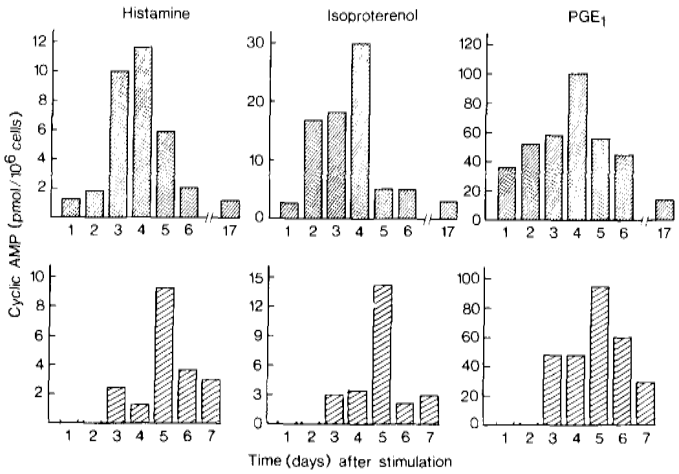

Figure 4. Regulation of T cell clones responsiveness to HA, Iso and PGE_1 as a function of time after cell activation. Clones 1E4 (*top panels*) and C4.2 (*lower panels*) were assayed on successive days after stimulation of cell proliferation by IL-2-containing media (day 0). Aliquots of cells removed each day were stimulated with HA (1.0 mM), Iso (0.01 mM), and PGE_1 (0.01 mM), and the resulting cAMP responses were measured as previously described.

therefore done by using clones alone without irradiated stimulator cells.

Additional experiments were performed to test whether the responses of cytolytic cells to PG and Iso also change after activation. Clones 1E4 and C4.2 were restimulated on day 0 with growth factor only without Ag, and the cAMP responses to the hormones were determined on subsequent days as previously described. As demonstrated in Figure 4, both clones responded to Iso and PGE_1, with increases in their cAMP levels as high as with cells stimulated with both Ag and IL-2. In addition, the Iso and PGE_1 responses peak on days 4 to 5 and then decline to the low basal levels, similar to the cellular responses to HA. Clone 1E4, when rested in culture without restimulation, retained this low but significant degree of hormonal responsiveness for at least 17 days. The cAMP curves of Figures 3 and 4 are representative of many such experiments, with the hormone response profiles paralleling cell activation/proliferation, as assessed by [^{3}H]TdR uptake or cell number. The peak hormone responsiveness is usually 1 day after peak proliferation, but this correlation between TdR incorporation and hormone responses is not precise. Thus, maximal hormone responses occur during or slightly after the period of maximal cell activation, but is not directly related to cell proliferation.

These results suggested that cell activation by itself, without stimulation by Ag, results in changes in responsiveness to all three hormones in a coordinate fashion. Several trivial explanations for these results have been examined. Cell population density, which increases during the course of the weekly growth cycle, has been shown to influence receptor expression in some cells (18, 19). When these clones were grown at different cell densities, however, there were no differences in the overall rise and fall in the levels of cAMP (on a per cell basis) attained after stimulation by these hormones. Similarly, dilution of dense cultures of postproliferative day 7 cells into fresh medium without IL-2 or Ag did not change their cAMP responses. To determine whether increases in the amount of cAMP in activated cells were due to increases in cell size associated with blastogenesis, cell volumes were measured in parallel with cAMP responsiveness. The mean cell volumes at the peak of hormonal responsiveness were actually lower than those earlier in the growth cycle (day 3; data not shown). These results demonstrate that the change in level of functional cyclase-linked hormone receptors is not simply due to increases in cell density or in cell size that accompany T cell activation and proliferation.

DISCUSSION

We have used cloned lymphocyte lines to define the repertoire of hormone receptors on defined subpopulations of T cells and to investigate the regulation of hormone responsiveness after cell activation. We herein demonstrate that all cytolytic clones tested have adenylate cyclase-linked surface receptors for HA, PG, and β-adrenergic agonists, but not for several other hormones. In addition, the presence of HA-R on one Th cell line and of all three receptors on unselected short term clones suggests that these receptors are present on a significant proportion of different T cells and are not just associated with the few Ag-specific Tc cells described here. Interestingly, the ability of these cells to respond to each hormone is modulated by cell activation. The hormone-stimulated levels of cAMP increase by day 2 after passage, peak on day 4 or 5, and then decline to basal levels of responsiveness as these cells reenter a postproliferative resting state.

Receptors for different hormones have been found on both mouse and human lymphocytes using a variety of techniques. Some hormone receptors, such as those for insulin, can be demonstrated by direct binding assays, whereas others, such as HA-R, are defined mainly by measuring the response of intracellular second messengers. The binding of HA H_2 agonists, β-adrenergic agents, and PG to lymphocytes bearing the appropriate receptors stimulates the activation of adenylate cyclase and causes a rapid increase in the intracellular concentration of cAMP. This rise in cAMP levels mediates the physiologic effects of these hormones in most systems, although some effects of HA may be caused by as yet unidentified mechanisms that do not involve H_1- or H_2-R (20). The immunologic effect of adenylate cyclase-activating hormones is often one of immunosuppression. HA and PGE_1 have been shown to inhibit the proliferative and effector

responses of T lymphocytes and NK cells (3, 9, 21). Both PG and HA appear to be involved in the induction of Ts cells (22–24). HA has the interesting property of being involved with two opposing processes, the induction of T cell suppression through activation of H_2-R and the activation of contrasuppression via H_1-R (24). Thus, the specific set of receptors expressed on the surface of a particular lymphocyte subset plays a critical role in determining the specific hormonal control mechanisms to which these cells are susceptible.

In many previous studies, the heterogeneous nature of the cell populations studied precluded the precise identification of the cells responding to these ligands. For example, any suppressive phenomenon observed in bulk populations in vitro could be due to a direct effect on the cells being measured (e.g., Tc cells) or could be mediated through effects of other cells (e.g., Th, Ts, or accessory cells). We have therefore characterized a set of hormone receptors on cloned populations of T lymphocytes in order to avoid indirect effects of extraneous cell populations. A similar approach by Kahn et al. (25) demonstrated HA and β-adrenergic receptors on several T cell lines, further demonstrating that a significant proportion of T cells bear receptors for these ligands.

The responses of these T cell clones to HA, PG, and Iso are mediated by typical H_2, PGE, and β_2-adrenergic receptors, respectively, as shown by the agonist/antagonist experiments. The rapid rise and fall of cAMP levels in response to HA, previously shown to involve receptor desensitization (10), is similar to the response kinetics seen with Iso, suggesting that similar desensitization occurs with these β-adrenergic receptors. This would be in keeping with previous demonstrations of β-adrenergic receptor desensitization in other systems (26, 27). The levels of cAMP reached after PG stimulation is not only higher, but also longer lasting, with little or no decrease noted for at least 1 h. This suggests that the physiologic signal transmitted to cells would be different for PGE_1 compared with that of HA or Iso.

After restimulation of quiescent cells, there is an increase in the clones' ability to respond to all three hormone agonists, with a subsequent decline as the cells cease proliferating. The previous demonstration of increased susceptibility of Tc cells to inhibition by HA with time after alloimmunization (28) may be related to this modulation of hormone responsiveness with cell activation. These changes in functional receptor activity, regardless of the mechanisms of such control, are analogous to the up-regulation of other receptors after lymphocyte activation. Stimulation of lymphocytes causes an increase in the number of surface receptors for a variety of ligands, including transferrin (29), insulin (1, 30, 31), and the lymphokine IL-2 (32). The increase in receptors for transferrin are presumably related to the changing metabolic and nutritional requirements associated with cell activation. The induction of growth factor and insulin receptors reflect a need for new immunoregulatory circuits to control the growth and effector activities of activated cells, which are not required for resting lymphocytes. For example, the increase in insulin receptors seen within 48 h of alloantigen stimulation initiates a mechanism for the augmentation of the developing cytolytic immune response (33). The increase in responsiveness to cAMP-active ligands would similarly enhance T cell sensitivity to the regulatory effects of these agents. The local release of these soluble mediators could then activate important negative feedback mechanisms to control the ongoing inflammatory and immune responses. The low level of responsiveness to HA, Iso, and PGE_1 persisting in unstimulated cells (Fig. 4) may indicate the presence of a minor tonic inhibition of immune responsiveness or, alternatively, may reflect the inability of T cell clones to return to a fully resting state (34).

The stimulation of Tc clones in IL-2-containing medium is sufficient to maximally induce the increased responses to HA, Iso, and PG, whereas the addition of specific Ag has no augmenting effect. This requirement for growth factor stimulation is in contrast to early events in T cell activation, such as up-regulation of IL-2R that are caused by stimulation of the Ag-R complex. Furthermore, by the time of maximal hormone responsiveness (4 to 5 days after passage), these T cell clone cultures are not synchronized with respect to the cell cycle. These collective observations suggest that the increased hormone responsiveness is a late result of cell activation per se and is not due to any relationship between hormone receptor activity and specific phases of the cell cycle. They further suggest that, if similar changes occur in fully resting T cells in vivo, they would have to take place after antigenic stimulation and the resulting induction of IL-2R expression and IL-2 secretion.

The precise mechanism by which these T lymphocytes modulate their responsiveness to HA, β-adrenergic agents, and PG after activation is not yet known. There are three major sites at which these changes could take place. 1) Cell activation could result in an increase in the number of surface receptors for each of these hormones, as occurs for insulin and IL-2. 2) An increase in the available C subunit could also give rise to enhanced conversion of ATP to cAMP. 3) An enhancement of the coupling between the hormone receptor and the C subunit could occur, which would increase the amount of C subunit activation by a given number of hormone-receptor complexes (35). The coupling between receptor and C subunit is mediated by an integral membrane guanine nucleotide binding protein (G_s) (26, 36). Changes in the amount or activity of G_s could alter receptor-cyclase coupling and thereby modulate the cAMP responses to these ligands. Preliminary experiments with forskolin (which directly activates the C subunit) and cholera toxin (an activator of G_s) showed a rise in stimulated cAMP levels in activated cells similar to the increased responses to HA, Iso, and PG. This suggests that there are changes in G_s or the C subunit that are responsible for the increases in cAMP responses, independent of any modulation that may take place in the expression of hormone receptors on the cell surface. Alterations in G_s or C subunit activities, membrane components that are common to all three hormone receptor-adenylate cyclase complexes, would explain the coordinate nature of the regulation of responsiveness to these agents after activation. Thus, we propose that T cell activation causes an elevation in G_s/C subunit activity that results in an increase in the intracellular cAMP concentrations stimulated by HA, β-adrenergic agents, and PG. Further experiments will be necessary to determine whether simultaneous changes also occur in surface hormone receptor levels after T cell activation.

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REFERENCES

1. **Krug, U., F. Krug, and P. Cuatrecasas.** 1972. Emergency of insulin receptors on human lymphocytes during in vitro transformation. *Proc. Natl. Acad. Sci. USA 69:2604.*
2. **Ottaway, C. A., and G. R. Greenberg.** 1984. Interaction of vasoactive intestinal peptide with mouse lymphocytes: specific binding and the modulation of mitogen responses. *J. Immunol. 132:417.*
3. **Bourne, H. R., L. M. Lichtenstein, K. L. Melmon, C. S. Henney, Y. Weinstein, and G. M. Shearer.** 1974. Modulation of inflammation and immunity by cyclic AMP. Science 184:19.
4. **Baker, P. E., J. V. Fahey, and A. Manck.** 1981. Prostaglandin inhibition of T cell proliferation mediated at two levels. *Cell. Immunol. 61:52.*
5. **Rocklin, R. E.** 1976. Modulation of cellular immune responses in vivo and in vitro by histamine receptor-bearing lymphocytes. *J. Clin. Invest. 56:1051.*
6. **Rappaport, R. S., and G. R. Dodge.** 1982. Prostaglandin E inhibits the production of human interleukin-2. *J. Exp. Med. 155:943.*
7. **Makoul, G. T., D. R. Robinson, A. K. Bhalla, and L. H. Glimcher.** 1985. Prostaglandin E_2 inhibits the activation of cloned T cell hybridomas. *J. Immunol. 134:2645.*
8. **Henney, C. S., H. R. Bourne, and L. M. Lichtenstein.** 1972. The role of cyclic 3′,5′ adenosine monophosphate in the specific cytolytic activity of lymphocytes. *J. Immunol. 108:1526.*
9. **Plaut, M., L. M. Lichtenstein, E. Gillespie, and C. S. Henney.** 1973. Studies on the mechanism of lymphocyte-mediated cytolysis. IV. Specificity of the histamine receptor on effector T cells. *J. Immunol. 111:389.*
10. **Schreurs, J., M. O. Dailey, and H. Shulman.** 1984. Pharmacologic characterization of histamine H_2 receptors on clonal cytolytic T lymphocytes: evidence for histamine-induced desensitization. *Biochem. Pharmacol. 33:3375.*
11. **Steiner, A. L., C. W. Parker, and D. M. Kipnis.** 1972. Radioimmunoassay for cyclic nucleotides. I. Preparation of antibodies and iodinated cyclic nucleotides. *J. Biol. Chem. 247:1106.*
12. **Dailey, M. O., C. G. Fathman, E. C. Butcher, E. Pillemer, and I. L. Weissman.** 1982. Abnormal migration of T lymphocyte clones. *J. Immunol. 128:2134.*
13. **Dailey, M. O., E. Pillemer, and I. L. Weissman.** 1982. Protection against syngeneic lymphoma by a long-lived cytotoxic T cell clone. *Proc. Natl. Acad. Sci. USA 79:5384.*
14. **Kimoto, M., and C. G. Fathman.** 1981. Antigen-reactive T cell clones. II. Unique homozygous and (high responder × low responder)F_1 antigen-presenting determinants detected using poly (Tyr,Glu)-poly D, L-Ala-poly Lys-reactive T cell clones. *J. Exp. Med. 153:375.*
15. **Brooker, G., J. F. Harper, W. L. Terasaki, and R. D. Moylan.** 1979. Radioimmunoassay of cyclic AMP and cyclic GMP. *Cyclic Nucleotide Res. 10:1.*
16. **Zick, Y., R. Cesla, and S. Shaltiel.** 1978. Non-hormonal burst in the level of cAMP caused by a "temperature shock" to mouse thymocytes. *FEBS Lett. 90:239.*
17. **Lands, A. M., A. Arnold, J. P. McAuliff, F. P. Ludens, and T. G. Brown.** 1967. Differentiation of receptor systems activated by sympathomimetic amines. *Nature 214:587.*
18. **Harden, T. K., S. J. Foster, and J. P. Perkins.** 1979. Differential expression of components of the adenylate cyclase system during growth of astrocytoma cells in culture. *J. Biol. Chem. 254:4416.*
19. **Pochet, R. D., D. A. Green, T. J. Goka, R. B. Clard, R. Barber, J. E. Dumont, and R. W. Butcher.** 1982. β-Adrenergic receptors and cyclic AMP responses to epinephrine in cultured human fibroblasts at various population densities. *J. Cyclic Nucleotide Res. 8:83.*
20. **Vickers, M. R., K. Millner, D. Martin, and C. R. Ganellin.** 1982. Histamine-induced inhibition of lymphocyte proliferation and lysosomal enzyme release from polymorphs may not be mediated via H_1- or H_2-receptors. *Agents Actions 12:5.*
21. **Katz, P., A. M. Zaytoun, and A. S. Fauci.** 1982. Mechanisms of human cell-mediated cytotoxicity. I. Modulation of natural killer cell activity by cyclic nucleotides. *J. Immunol. 129:287.*
22. **Fulton, A. M., and J. G. Levy.** 1981. The induction of nonspecific T suppressor lymphocytes by prostaglandins E_1. *Cell. Immunol. 59:54.*
23. **Chouaib, S., L. Chetenoud, D. Klatzmann, and D. Fradelizi.** 1984. The mechanisms of inhibition of human IL-2 production. II. PGE_2 induction of suppressor T lymphocytes. *J. Immunol. 132:1851.*
24. **Seigel, J. N., A. Schwartz, P. W. Askenase, and R. K. Gershon.** 1982. T-cell suppression and contrasuppression induced by histamine H_2 and H_1 receptor agonists, respectively. *Proc. Natl. Acad. Sci. USA 79:5052.*
25. **Khan, M. M., K. L. Melmon, C. G. Fathman, B. Hertel-Wulff, and S. Strober.** 1985. The effects of autacoids on cloned murine lymphoid cells: modulation of IL 2 secretion and the activity of natural suppressor cells. *J. Immunol. 134:4100.*
26. **Sibley, D. R., and R. J. Lefkowitz.** 1985. Molecular mechanism of receptor desensitization using the β-adrenergic receptor-coupled adenylate cyclase system as a model. *Nature 317:124.*
27. **Harden, T. K.** 1983. Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase. *Pharmacol. Rev. 35:5.*
28. **Plaut, M., L. M. Lichtenstein, and C. S. Henney.** 1975. Properties of a subpopulation of T cells bearing histamine receptors. *J. Clin. Invest. 55:856.*
29. **Haynes, B. F., M. Hemler, T. Cotner, D. L. Mann, G. S. Eisenbarth, J. L. Strominger, and A. S. Fauci.** 1981. Characterization of a monoclonal antibody (5E9) that defines a human cell surface antigen of cell activation. *J. Immunol. 127:347.*
30. **Heidermann, J. H., T. C. Reynolds, and T. B. Strom.** 1978. The insulin receptor as a universal marker of activated lymphocytes. *Eur. J. Immunol. 8:589.*
31. **Braciale, V. L., J. R. Gavin, III, and T. J. Braciale.** 1982. Inducible expression of insulin receptors on T lymphocyte clones. *J. Exp. Med. 156:664.*
32. **Helmes, M. E., M. B. Brenner, J. M. McLean, and J. L. Strominger.** 1984. Antigenic stimulation regulates the level of expression of interleukin 2 receptor on human T cells. *Proc. Natl. Acad. Sci. USA 81:2172.*
33. **Strom, T. B., R. A. Bear, and C. B. Carpenter.** 1975. Insulin induced augmentation of lymphocyte-mediated cytotoxicity. *Science 187:1206.*
34. **Dailey, M. O., W. M. Gallatin, and I. L. Weissman.** 1985. The in vivo behavior of T cell clones: altered migration due to loss of the lymphocyte surface homing receptor. *J. Mol. Cell. Immunol. 2:27.*
35. **Beckman, B. S., and M. D. Hollenberg.** 1979. Beta-adrenergic receptors and adenylate cyclase activity in rat reticulocytes and mature erythrocytes. *Biochem. Pharmacol. 28:239.*
36. **Rodbell, M.** 1980. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature 284:17.*