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BETA-ADRENERGIC RECEPTORS ON HUMAN SUPPRESSOR, HELPER, AND CYTOLYTIC LYMPHOCYTES*

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Abstract—Using the radioligand beta-adrenergic blocker [^{125}I]cyanopindolol (ICYP), we have characterized the beta-adrenergic receptors on Leu 3$^+$ (T helper [T_H]), Leu 2$^+$, 9.3$^-$ (T suppressor [T_s]) and Leu 2$^+$, 9.3$^+$ (T cytolytic [T_c]) subsets of human lymphocytes. Peripheral blood T cells were isolated by rosetting, and then subsets were purified by their affinities to monoclonal antibodies against their Leu 3 and 9.3 markers. ICYP binding to the subsets was saturable with time and with concentration; the binding was stereoselective and reversible by beta-adrenergic antagonists. A biological response produced by beta agonists increased intracellular concentrations of cAMP and corresponded to the number of binding sites. Each subset of cells had a number of binding sites, which was characteristic for the given subset. The data indicate that the density of distribution of beta-adrenergic receptors was not homogeneous on the precursors of phenotypically and functionally distinct T cells ($T_s \sim 2900$, $T_c \sim 1800$ and $T_H \sim 750$ binding sites). The displacement studies using beta-adrenergic agonists were performed on the cytolytic and suppressor T cell subsets, suggesting that the receptors were mainly of the beta-2 type. The immunobiological significance of such selective distribution of numbers and subtypes of beta-adrenergic receptors on distinct T cell subsets is under investigation.

Catecholamines are modulators of *in vivo* and *in vitro* models of immunity [1]. For example, isoproterenol can inhibit antibody production [2, 3] and PHA¶-induced lymphocyte blast transformations as measured by tritiated thymidine uptake [2]. A number of beta-adrenergic agents have been reported to inhibit the function of cytolytic T cells, the release of lysosomal hydrolases from human neutrophils, and the release of mediators from mast cells [1–7]. Most of these studies were performed on mixtures of cells and did not permit distinction of precisely which cells the agonists stimulated to modulate their function. The data show that such catecholamine-induced changes in the activity of the lymphoid cells are via classical cell surface specific receptors. The intracellular effects in each instance have been proportional to the intracellular accumulation of cyclic AMP.

T lymphocytes are both regulatory and effector cells in the immune response. In recent years, monoclonal antibodies have been used to identify T cell surface glycoproteins which, in turn, have been used to distinguish T lymphocyte subsets with different functional repertoires. Initial studies [8, 9] in a number of laboratories defined two mutually exclusive subsets of human T cells. The suppressor/cytotoxic (CD8) subset defined by anti-Leu 2 or OKT8 antibodies comprises approximately 30% of peripheral T cells in healthy individuals. Included within the CD8 subset are both cytotoxic and suppressor cells, which interact with their targets in a class I (HLA-A,B) restricted manner. A reciprocal subset (designated CD4) defined by anti-Leu 3 or OKT4 antibodies, represents approximately 60% of peripheral T cells in healthy individuals and is designated the helper/inducer subset. Signals from this subset as well as foreign antigen are required for B cells to differentiate into plasma cells and secrete immunoglobulin. CD4$^+$ cells also induce the differentiation of other functional subsets of T cells such as cytotoxic and suppressor cells. Helper/inducer cells are activated by antigen-presenting accessory cells only if the latter cells express class II HLA antigens (for example, HLA-DR) that are identical to that of the helper cell. Moreover, recent studies indicate that some CD4$^+$ cells can differentiate into cytotoxic T cells but, unlike CD8$^+$ killer cells, such CD4$^+$ cells kill with specificity for class II HLA molecules of the target cells [10]. Thus, CD4$^+$ helper/inducer cells and cytolytic cells, like CD8$^+$ cells, must recognize both foreign antigen and self-HLA determinants but, unlike CD8$^+$ cells, CD4 cells respond to antigen in association with class II HLA determinants [8].

We have reported recently [11] that beta-adrenergic receptors are nonrandomly distributed on the precursors of phenotypically and functionally distinct human T cell subsets. The pharmacologic characteristics of the beta-adrenergic receptors on such

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¶ Abbreviations: PHA, phytohemagglutinin; cAMP, adenosine 3′,5′-monophosphate; SRBC, sheep erythrocytes and ICYB, [^{125}I]cyanopindolol.

subsets of T cells were not investigated. In this paper, we quantify and characterize the beta-adrenergic receptors on the surface of human suppressor (Leu $2^+, 9{\cdot}3^-$), cytolytic (Leu $2^+, 9{\cdot}3^+$), and helper (Leu 3^+)T cells.

MATERIALS AND METHODS

Pharmacological agents. [^{125}I]Cyanopindolol, used for these studies, had a specific activity of >2000Ci/mmole and was purchased from the Amersham Corp. (Arlington Heights, IL). Other compounds used in this study were: (−)-isoproterenol, (−)-epinephrine, (−)-norepinephrine, *dl*-propranolol (all from the Sigma Chemical Co., St. Louis, MI) and *d*-propranolol and *l*-propranolol (Ayerst Laboratories, New York, NY).

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 [12, 13]. Antibody 9.3 was provided by Dr. J. A. Hansen, Fred Hutchinson Cancer Research Center, Seattle, WA. Characteristics of cells possessing 9.3^+ and 9.3^- phenotypes have been described [8, 11, 14].

Isolation of E-rosetting lymphoid cells. Peripheral blood mononuclear cells from healthy volunteers were isolated by Ficoll-Hypaque centrifugation of fresh defibrinated blood [15]. T cells were separated from others by a single step rosetting method [16] using 2-aminoethyl isothiouronium bromide hydrobromide (Sigma) treated sheep erythrocytes (AET-SRBC). Rosette forming T cells were separated on a second Ficoll-Hypaque gradient and freed from SRBC by hypotonic lysis. The final T cell fraction contained more than 95% of the E-rosette forming cells. The non-T cell fraction included 49–50% surface Ig positive B cells, 30–50% alpha napthyl acetate esterase positive phagocytic, plastic-adherent monocytes, and 5–10% non-adherent nonphagocytic surface Ig negative, non-T non-B cells.

Isolation of subsets of T cells by the panning technique. T cell subsets were obtained by a panning technique [13] which permitted the fractionation of freshly obtained E-rosette forming cells (T cells) into Leu 2 and Leu 3 subpopulations. Briefly, 3×10^7 unfractionated T cells were treated for 20 min with 0.5 ml antibody-containing supernatant fractions, washed twice in 5% fetal calf serum-phosphate buffered saline (FCS-PBS), and transferred into 15 × 100 mm petri dishes (Lab-Tek, Naperville, IL) that were coated previously with affinity-purified goat anti-mouse IgG at 10 μg/ml in 0.05 M Tris buffer, pH 9.5. The dishes were incubated at 4° for 2 hr. The non-adherent cells were then decanted, and the dishes were gently washed five times with 1% FCS-PBS. Then the bound cells were recovered by vigorous pipetting. $CD4^+$ and $CD8^+$ cells isolated with this technique contained 97 ± 3% T cells of the appropriate subset as determined by cell sorter analysis. From 90 ml of blood ($\simeq 100 \times 10^6$ cells) approximately $50–80 \times 10^6$ T cells, $\simeq 26 \times 10^6$ Leu 2^-, $\simeq 10 \times 10^6$ Leu 2^+, $9{\cdot}3^-$ and $\simeq 16 \times 10^6$ Leu 2^+, $9{\cdot}3^+$ cells were obtained.

Measurement of [^{125}I]*CYP binding.* Binding experiments were performed by incubating 6×10^5 cells with different concentrations of [^{125}I]CYP in a total volume of 300 μl of incubation buffer (20 mM Tris, 150 mM saline and 1 mM ascorbic acid, pH 7.4) for 90 min at 30°. In the competition experiments, various concentrations of agonists or antagonists were added to the incubation as indicated. Incubations were terminated by rapidly diluting them with 5 ml of incubation buffer at room temperature followed by vacuum filtration of the diluted incubate through GF/C Whatman glass fiber filters. The filters were washed with 30 ml of the incubation buffer at room temperature. After being dried, the filters were counted in a Micromedic Systems automatic gamma counter.

In each experiment, the nonspecific binding to cells was determined by measuring the amount bound when the cells were incubated in the presence of 6×10^{-6} M *dl*-propranolol. Analysis of the relationship between bound (B) and free (F) ligand at equilibrium was done by graphic representation. The Scatchard plot allowed estimation of the K_D, maximum agonist bound, and density of receptors/cell in the subsets.

RESULTS

Binding characteristics of [^{125}I]*CYP to intact suppressor, cytolytic and helper T cells.* In these assays, subsets of lymphoid cells were incubated for 90 min at 30° in the presence of increasing concentrations of [^{125}I]CYP (3–200 pmoles). We chose *dl*-propranolol as a beta-adrenergic blocking agent at a concentration of 6.0 μM for estimating nonspecific binding of [^{125}I]CYP. Specific binding was expressed as the difference between counts bound in the absence and presence of 6 μM *dl*-propranolol. Specific binding achieved equilibrium over a 60–75 min period. This time course yielded a rate of

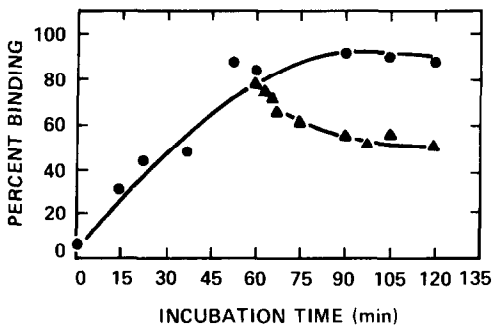

Fig. 1. Time course of binding of [^{125}I]CYP (●) and reversal of binding of [^{125}I]CYP by 6 mM *dl*-propranolol (▲). In this experiment 6×10^5 cells were incubated with [^{125}I]-CYP (50 μM) in the absence and presence of 6 μM *dl*-propranolol. Specific binding, i.e. the difference between counts bound in the absence and presence of 6 μM *dl*-propranolol, was determined in triplicate at the given time. The second rate constant, K_1 was calculated from the equation $(K_{ob} - K_2)/[^{125}\text{I}]\text{CYP}$, where K_2 is the rate constant for the reversal of binding (dissociation constant). For the reversal of binding (▲), 6×10^5 cells were incubated with [^{125}I]CYP (50 pM) for 45 min. *dl*-Propranolol was added to a final concentration of 6.0 μM, and specifically bound counts were determined in triplicate at the given time.

association (K_1) of $2.52 \times 10^8\,M^{-1}\,min^{-1}$ (T suppressor cells) (Fig. 1). Reversal of [^{125}I]CYP binding by adding 6.0 μM *dl*-propranolol indicated that specific binding reversed with a dissociation constant (K_2) of $0.0031\,min^{-1}$ (Fig. 1). In suppressor T cells, binding of increasing concentrations of [^{125}I]CYP with intact cells was saturable (Fig. 2) The K_D of binding was $2.5 \pm 0.4 \times 10^{-11}$ M (mean, N = 5) and the number of binding sites per cell was ~2900 ± 201. Scatchard analysis indicated a single class of binding sites. In cytolytic T cells, the K_D of binding was $5.2 \pm 0.8 \times 10^{-11}$ M (mean, N = 4) and the number of binding sites per cell was ~1800 ± 142 (Fig. 2). In helper T cells, binding of increasing concentrations of [^{125}I]CYP with intact cells was saturable (Fig. 2). The dissociation constant of binding calculated by Scatchard analysis was $2.5 \pm 0.6 \times 10^{-11}$ M (mean, N = 4) and the number of binding sites per cell was ~750 ± 64.

Displacement of [^{125}I]CYP binding by d- *and* l-*propranolol in suppressor and cytolytic T cells.* Beta-adrenergic receptors exhibited stereoselectivity in expressing their activities. As shown in Fig. 3, the beta-adrenergic antagonists *d*- and *l*-propranolol competed for the binding sites on suppressor T cells. On suppressor T cells, *l*-propranolol was approximately 150-fold more potent than *d*-propranolol in inhibiting [^{125}I]CYP binding. The estimated value for the dissociation constant was $3.0 \pm 0.4 \times 10^{-11}$ M for *l*-propranolol and $5.0 \pm 0.2 \times 10^{-9}$ M for *d*-propranolol. In cytolytic T cells, *l*-propranolol was approximately 30-fold more potent than *d*-propranolol in inhibiting [^{125}I]CYP binding. The K_D values were $4.0 \pm 0.3 \times 10^{-10}$ M and $1.2 \pm 0.06 \times 10^{8}$ M for *l*- and *d*-propranolol respectively (Fig. 3). These studies were not performed with helper T cells because net specific binding counts were not high enough for such a study.

Competition for [^{125}I]CYP binding sites by beta-adrenergic agonists. Three beta-adrenergic agonists, (−)-isoproterenol, (−)-epinephrine, and (−)-norepinephrine, were tested for their abilities to inhibit [^{125}I]CYP binding on suppressor and cytolytic T cells. The beta-adrenergic agonists competed for the [^{125}I]CYP binding sites on suppressor and cytolytic (Fig. 4) T cells with an order of potency of (−)-isoproterenol > (−)-epinephrine > (−)-norepinephrine. The displacement obtained with three beta-adrenergic agonists suggested that the receptor sites on suppressor and cytolytic T cells were mainly of the beta-2 type. The derived K_D values were approximately 5 ± 1.2 μM for (−)-isoproterenol, 40 ± 10 μM for (−)-epinephrine and 100 ± 10 μM for (−)-norepinephrine on suppressor T cells (N = 6). The only exception to these results was one in the lymphocytes of a healthy volunteer where a dissociation constant of 250 nM was obtained for isoproterenol on suppressor T cells. The derived K_D values for cytolytic T cells were approximately 12 ± 3 μM for (−)-isoproterenol, 50 ± 10 μM for (−)-epinephrine and 400 ± 150 μM for (−)-norepinephrine (N = 5). There was one exception among cytolytic cells of another healthy volunteer where a dissociation constant of 700 nM was obtained for (−)-epinephrine. Due to low net binding, the agonist-mediated displacement experiments were not performed with helper T cells. A summary of dissociation constants for various beta-receptor agonists and propranolol is shown in Table 1.

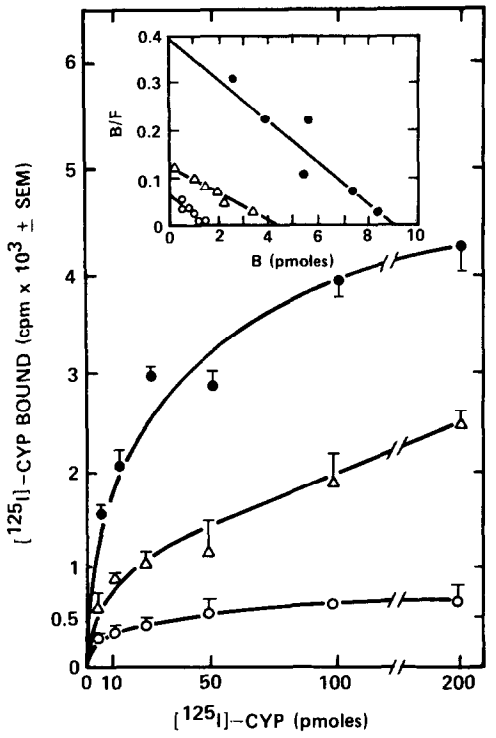

Fig. 2. Specific [^{125}I]CYP binding to human suppressor (●) helper (○), and cytolytic (△) T cells as a function of concentration. Cells (6×10^5) were incubated with increasing concentrations of [^{125}I]CYP in the Tris, saline and ascorbic acid buffer (pH 7.4) at 30° for 90 min, and the specific binding was calculated for each concentration of [^{125}I]-CYP. The figure shows one representative experiment from five different experiments; each point is the average of triplicates. The insert shows a Scatchard plot of [^{125}I]CYP binding. For suppressor cells, $K_D = 2.5 \pm 0.4 \times 10^{-11}$ M, number of binding sites per cell ~ 2900 ± 201 (N = 5). For cytolytic cells, $K_D = 5.2 \pm 0.8 \times 10^{-11}$ M, number of binding sites per cell ~ 1800 ± 142 (N = 4). For helper cells, $K_D = 2.5 \pm 0.6 \times 10^{-11}$ M, number of binding sites per cell ~ 750 ± 64 (N = 4).

DISCUSSION

This study confirms that immunologically uncommitted subsets of T cells generally possess beta-adrenergic receptors on their cell surface. Our use of monoclonal antibodies to specific cell surface markers made it possible to separate the precursors of helper, suppressor, and cytolytic T cells into immunologically, relatively homogeneous populations.

Beta-adrenergic receptors on human lymphocytes have been characterized in previous reports only in heterogeneous mixtures of the cells [17–19]. Our study examines the complement and characteristics of these beta-adrenergic receptors in phenotypically and functionally distinct subsets of human T cells. This study was undertaken because recent obser-

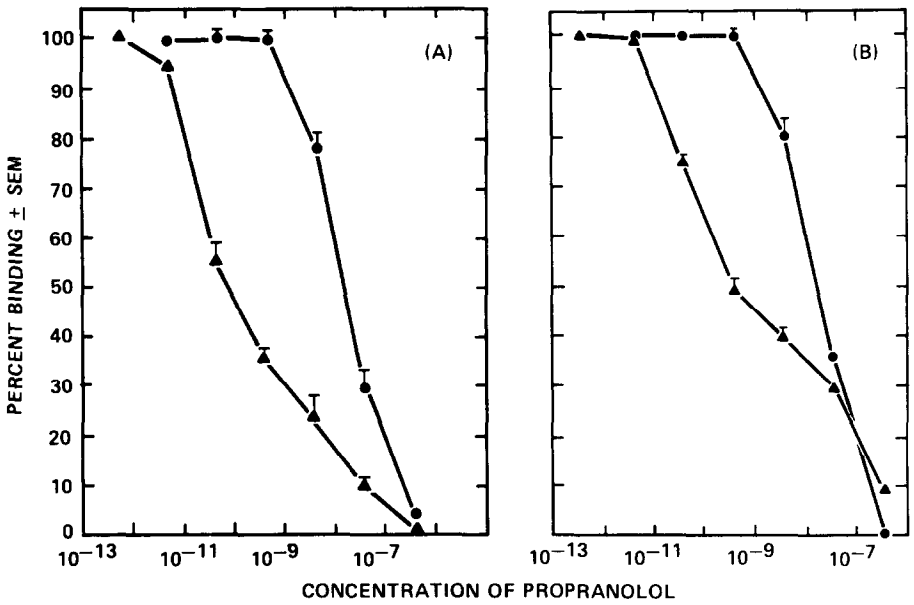

Fig. 3. Reversal of [^{125}I]CYP binding by *d*- and *l*-propranolol in suppressor (A) and cytolytic (B) T cells. Intact cells were incubated with [^{125}I]CYP (50 pM) in the presence of the given concentrations of *l*- (▲) and *d*- (●) propranolol for 60 min. Binding was determined as described. Results plotted are values derived from triplicate determinations in at least three experiments.

vations have demonstrated that "average" responses in cell mixtures do not reflect the specific roles of response of subsets of T cells [11]. When the cells are separated, studied independently, and systematically recombined, the sequence and priority of autacoid modulation of immunity begin to emerge, and the cell–cell control of response to the modulators becomes apparent. Based on our observations we reasoned that the individual T cell receptor complements could not necessarily be predicted from the response of mixtures, and that one mechanism of possible cellular individuality of response to autacoids may lie in the numbers and characteristics of the receptors on the different subsets. This study proves that the density and distribution of beta-adrenergic receptors are distinct in phenotypically

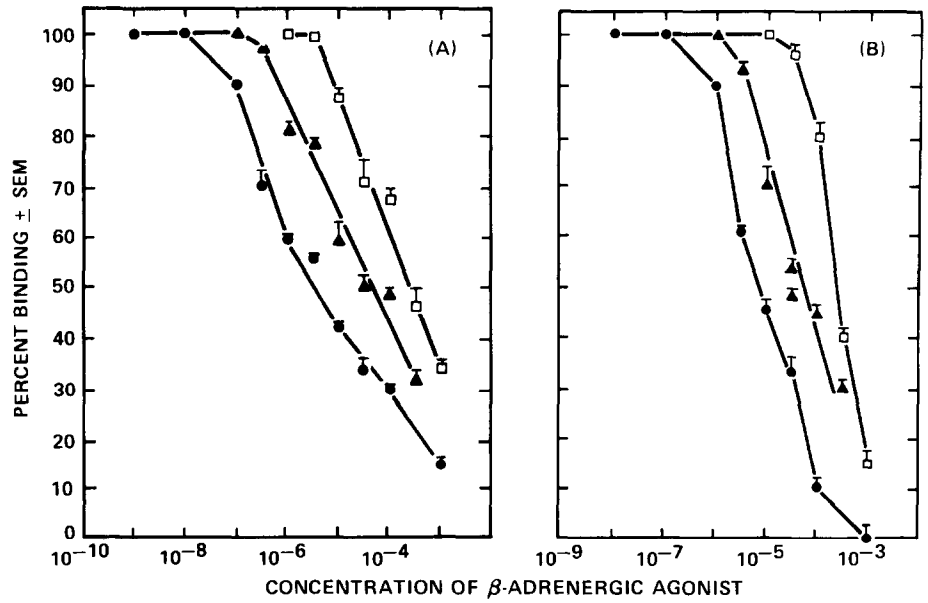

Fig. 4. Competition for [^{125}I]CYP binding sites by beta-adrenergic agonists on suppressor (A) and cytolytic (B) T cells. Various concentrations of (−)-isoproterenol (●), (−)-epinephrine (▲) and (−)-norepinephrine (□) were incubated with cells in the presence of [^{125}I]CYP for 60 min. The data shown are from one representative experiment of four experiments performed in duplicate.

Table 1. Apparent dissociation constants of beta-adrenergic agents

Beta-adrenergic agonist or antagonist	Dissociation constant (K_D) [^{125}I]CYP binding			
	T_s (N = 6)*		T_c (N = 5)†	
d-Propranolol	5 ± 0.2	nM	12 ± 0.6	nM
l-propranolol	30 ± 4	pM	400 ± 30	pM
(−)-Isoproterenol	5 ± 1.2	μM	12 ± 3	μM
(−)-Epinephrine	40 ± 10	μM	50 ± 10	μM
(−)-Norepinephrine	100 ± 10	μM	400 ± 150	μM

Values are means ± S.E.M.

* The cells of one healthy volunteer with a K_D of 250 nM for isoproterenol were not included in this group.

† The cells of another healthy volunteer with a K_D of 700 nM for epinephrine were not included in this group.

and functionally distinct subsets of T cells. We have also reported a distinct distribution of beta-adrenergic receptors on cloned murine lymphoid cells and on subsets of human helper T cells [20, 21].

Williams *et al.* [17] first demonstrated specific beta-adrenergic receptors on membrane preparations of unseparated total lymphocytes. Using [^{3}H]-alprenolol as the marker for the receptor, they concluded that there were approximately 2000 receptors per cell. Our data indicate approximately 2900 receptor sites per T_s cell, 1800 receptor sites per T_c cell and 750 receptor sites per T_H cell. Each subset has statistically significant ($P < 0.05$) different numbers of receptors than the other two subsets.

The binding sites on suppressor and cytolytic cells showed the saturability and stereospecificity expected for true beta-adrenergic receptors. The specific binding of [^{125}I]CYP on suppressor, cytolytic, and helper cells was a saturable process, indicating the existence of a finite number of receptor sites per cell. The stereospecificity was clearly demonstrated by using *l*- and *d*-propranolol as beta-adrenergic antagonists for inhibition studies on suppressor and cytolytic T cells. The dissociation with three agonists demonstrated the presence of a majority of beta-$_2$ receptors [(−)-isoproterenol > (−)-epinephrine > (−)-norepinephrine]. This order of potency is in agreement with the report of Williams *et al.* on total lymphocytes [17]. Our binding experiments of T cell subsets were conducted with cells having different antibodies on them. While they may have exerted some undetected effect on binding, the antibodies did not affect cAMP responses (unpublished observation).

We have reported recently on basal cAMP levels and response to isoproterenol in precursor as well as mitogen-stimulated helper, cytolytic, and suppressor subsets of T cells [11]. We found that the rank order of basal cAMP levels was ($T_c > T_s > T_h$) but that the rank order of maximal response to isoproterenol was different. T_s cells (Leu 2$^+$, 9·3$^-$) were more responsive than T_c cells (Leu 2$^+$, 9·3$^+$) to isoproterenol, the latter were more responsive than T_H cells (Leu 3$^+$) [11]. We conclude that the beta-receptor density on each cell is proportional to and is predictive of the maximal cAMP generated.

Activation of the subsets with mitogen (PHA or Con A) does not alter the basal or isoproterenol-stimulated cAMP concentration [11], suggesting that mitogen stimulation of immunologically uncommitted subsets of T cells does not alter the expression of beta-adrenergic receptors as it does for histamine receptors on T_s and T_c cells [11].

The distinctly different quantitative beta-adrenergic receptor distribution among subsets suggests that the autacoid may uniquely modulate the cyclic AMP production of individual subsets. If the biologic functions of the receptors were all proportionate to the absolute concentration of cAMP or to the number of agonist-occupied receptors, the receptor distribution would account for distinct cell functions in given concentrations of agonist. However, the modulatory roles of biologic functions of these subsets have not yet been investigated thoroughly. Therefore, the role of receptor complement in the characteristic biologic functions of the subsets cannot be determined.

Our study of the binding of a radioligand ([^{125}I]-CYP) to beta-adrenergic receptors has enabled us to define several criteria for identifying classical beta-receptors on all major subsets of human lymphoid cells. These criteria include saturation of binding with time and ligand concentration, stereoselectivity, rank order of subsets to one biological response [11] that corresponds to radioligand binding, and reversibility of binding. The immunobiological significance of such selective distribution of beta-adrenergic receptors is under investigation.

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