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Immunology

T-cell suppression and contrasuppression induced by histamine H_2 and H_1 receptor agonists, respectively

(cytotoxic T lymphocyte/suppressor cell/contrasuppressor cell)

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ABSTRACT **The intensity of $Ly1^+$ T helper and delayed type hypersensitivity effector cell activities is governed, in part, by an interplay between two classes of immunoregulatory T cells: suppressor cells and contrasuppressor cells. We asked whether histamine, at concentrations and duration of exposure that we calculated might be achieved at local sites of inflammation, could activate either or both of these classes of regulatory cells *in vitro*. To answer this question we used spleen cells from mice treated *in vivo* with the toleragen trinitrobenzenesulfonic acid as regulators of *in vitro* generation of primary anti-trinitrophenyl self-cytotoxic T lymphocytes. Under the conditions used, these spleen cells had no major regulatory effects. However, if these cells were preincubated with histamine at 0.1 mM for 30–60 min, suppressor activity was induced, but this occurred inconsistently and with nonstoichiometric results. The use of synthetic histamine agonists revealed that histamine may activate both suppressor and contrasuppressor cell subsets. A histamine H_1 receptor agonist [2-(2-pyridyl)-ethylamine dihydrochloride] had a propensity to activate contrasuppression, whereas an H_2 receptor agonist (dimaprit) tended to activate suppressor cells. Thus, histamine may have opposing actions that obscure suppression. This duality was shown by treatment of pyridylethylamine-induced contrasuppressor cells with complement and anti-I-J antibody that kills contrasuppressor cells. This treatment revealed a high level of suppressor cell activity that was not expressed until the opposing contrasuppressor cells were removed. Because histamine is released at local sites of delayed type hypersensitivity, these results indicate that histamine may serve as an inducer of microenvironmental immunomodulation by activating regulatory T cells at sites where immune responses are taking place.**

The modulation of immune responses is brought about, in part, by sets of regulatory T cells. Suppressor T cells exert a negative regulation; removal of these cells often results in marked augmentation of immune responses (1). Another class of immunoregulatory T cells exert positive regulation in that they oppose suppressor cell activity. They have been named "contrasuppressor cells" and their activity has been referred to as "contrasuppression" (2, 3).

The observation that a population of cells had the capacity to block suppressor T cell activity suggested that such cells would be optimally suited to provide microenvironmental immunoregulation. Thus, one could hypothesize that, if a mediator that preferentially activated contrasuppressor cells were present at a local site, a heightened local response would result without necessarily altering the level of systemic immunity.

These considerations led us to look for possible endogenous mediators that could induce this postulated form of microenvironmental immunoregulation. We chose histamine as a substance to study, for two reasons: (*i*) functional histamine receptors have been reported to be present on immunoregulatory T cells (4–7); and (*ii*) mast cells, which contain significant quantities of histamine, degranulate at local sites of delayed type hypersensitivity responses (8). This degranulation should result in significant histamine release, leading transiently to high local levels of histamine. It is important to note that, in the mouse, serotonin is the principal vasoactive amine and histamine has no significant vasoactive effects (9). Thus, histamine released from mast cells should not affect local vascularity and may be specific as a microenvironmental mediator of immune modulation.

We tested the possibility that histamine could modulate an *in vitro* immune response. The model we studied was the *in vitro* production of killer cells specific for trinitrophenyl (TNP) modified self antigens. Mice that are rendered tolerant to picryl chloride contact hypersensitivity by intravenous injection of trinitrobenzenesulfonic acid (TNBS) (10) or of TNP-labeled syngeneic cells (11–13) develop suppressor T cells that can interfere with the adoptive transfer of contact hypersensitivity to nonimmune recipients (10) or with the generation of antigen-specific helper cells (12, 13). In spite of the presence of these suppressor T cells it has been difficult to demonstrate their action by *in vitro* "cell mixing" experiments using cell-mediated lympholysis (CML) as an assay system (11–13). In fact, in some instances not only are suppressor cells difficult to demonstrate in the TNP killer cell system but, when the cells from mice that have been rendered tolerant as judged by contact hypersensitivity responses are placed *in vitro*, killer cells actually develop (10).

This is not true in all mouse strains. $B6AF_1$ mice do not develop these killer cells after tolerization, so their cells could be used as a source of immunoregulatory cells because they themselves would not develop into killer cells. Thus, we had a model system in which suppressor T cells were probably present but their activity was difficult to demonstrate. We tested whether histamine could modulate the suppressor cells that inhibit contact hypersensitivity responses but fail to affect the *in vitro* development of TNP killer cells.

MATERIALS AND METHODS

Mice. Six- to 8-week-old $B6AF_1$ mice were obtained from The Jackson Laboratory and were allowed to rest for at least 1 week.

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Abbreviations: CML, cell-mediated lympholysis; CTL, cytotoxic T lymphocytes; PEA, 2-(2-pyridyl)ethylamine dihydrochloride; TNBS, trinitrobenzenesulfonic acid; TNP, trinitrophenyl.

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Drugs. Histamine (Sigma), 2-(2-pyridyl)ethylamine dihydrochloride (PEA; a histamine H_1 receptor agonist), and dimaprit (a histamine H_2 receptor agonist) (Smith Kline & French, Hertfordshire, England) were dissolved in phosphate-buffered balanced salt solution containing 5% fetal calf serum <2 hr before use and then sterilized by filtration through a 0.22-μm Millex filter (Millipore). If necessary, the pH was adjusted by using 1 M NaOH with phenol red dye as a visual indicator.

Generation of Cytotoxic T Lymphocytes (CTL). Responder spleen cells were suspended in the buffered balanced salt solution/calf serum mixture and cultured with TNP-labeled stimulator cells prepared in the following manner. Erythrocytes were lysed (14), washed twice, and treated with mitomycin C (Sigma; 30 μg/ml for 2.5×10^7 cells per ml, 37°C, 50 min). The cells then were washed twice in serum-free balanced salt solution; the pellet was resuspended in 1 ml of 10 mM TNBS (Eastman), and the cells were incubated for 10 min. Then, 10 ml of balanced salt solution/serum was added and the cells were washed three times.

Regulatory Cells. Mice syngeneic to responders were injected intravenously with 0.2 ml of TNBS (20 mg/ml) at 7 and 4 days before harvest for culture (10). On the day of culture, spleen cell suspensions were treated with mitomycin C, washed three times, and then suspended ($0.5–3 \times 10^7$ cells in 3–5 ml) in solutions of various histamine agonists and incubated for 60 min at 37°C without swirling. Finally, cells were washed three times and added to CML cultures to test their effect on killer cell generation.

CML Cultures. Viable responder cells (5×10^6) were cultured with 5×10^5 stimulator cells (15, 16). Each group consisted of three 2-ml tissue culture wells plated identically. Plates were incubated in a 5% CO_2/95% air in a humidified incubator at 37°C.

Assay for CTL. CML cultures were harvested after 5 days. The three wells representing one group were harvested, pooled, washed once in balanced salt solution/serum, and suspended to 0.8 ml. In all experiments, recovery of viable cells was 40–70%. Aliquots of culture cell suspension—0.1 ml (1/8 of culture) or 0.1 ml of a 1:3 or 1:9 dilution—were plated in quadruplicate in 96-well tissue culture plates (Falcon). Then, 0.1 ml of the ^{51}Cr-labeled target cell suspension (2×10^5 cells per ml) was added; the plates were sealed, centrifuged at 1,200 rpm for 4 min, and incubated at 37°C for 3.5–4.5 hr. Then the plates were vortexed vigorously for 30–60 sec to disrupt dead cells and centrifuged for 6 min at 1,200 rpm. A 0.1-ml aliquot of the supernatant was carefully removed and placed in a plastic tube for measurement of radioactivity. Calculation of percentage specific cytotoxicity was as described (15, 16). Data represent cytotoxicity at an effector/target cell ratio of 40:1. Cytotoxicity is expressed as the mean (± SD) of four identical wells calculated from samples derived from pooled wells. Background values were 20–40% of maximal release. The range of cytotoxicity in control groups was 25–65%. Statistical significance was calculated by the Student *t* test.

Preparation of Targets for TNP-Specific CTL. Two days before the assay, peritoneal exudate cells were induced by injection of 2 ml of sterile thioglycolate intraperitoneally into mice syngeneic to CTL. On the day of assay, the cells were harvested with phosphate-buffered saline. If necessary, erythrocytes were lysed. Then, 0.1 ml of $Na_2{}^{51}CrO_4$ (1 mCi/ml; 1 Ci = 3.7×10^{10} becquerels; New England Nuclear) was added. The cells were incubated in a 37°C waterbath for 50 min, washed twice in serum-free balanced salt solution, and coupled with TNBS as described above. The cells were then washed three times, and the viable cells were counted and brought to 2×10^5 cells per ml for plating.

Treatment of Regulatory Cells with Anti-I-J Antibody. Cells from $B6AF_1$ mice injected on day 7 and day 4 with TNBS were harvested, treated with mitomycin C, washed, preincubated with medium or 0.1 mM PEA for 1 hr at 37°C, and exposed to medium or a 1:20 dilution of anti-I-J^b and then to rabbit complement at a dilution of 1:16. After washing, 2.5×10^5 of these cells were added to $B6AF_1$ anti-TNP-$B6AF_1$ CML cultures and then, 5 days later, CTL were assayed with ^{51}Cr-labeled target TNP cells.

RESULTS

Tolerant Cells Express No Suppressive Activity Without a 1-Hr Incubation with Histamine. After determining that intravenous pretreatment of $B6AF_1$ mice with TNBS abolished their ability to make CTL in a 5-day CML culture (results not shown), we tested whether these nonresponsive cells could transfer nonresponsiveness to normal cells. We added varying numbers of "regulatory cells" obtained from mice tolerized *in vivo* with TNBS to 5×10^6 normal $B6AF_1$ spleen cells in a standard CML assay. The tolerant cells had been pretreated with mitomycin C to ensure that no killer cells would develop after their reaction with cells in a normal population. The regulatory cells from mice that had been made tolerant with TNBS did not affect the level of cytotoxicity generated by normal spleen cells (Fig. 1, bar B). However, if the regulatory cells were incubated for 1 hr in culture medium containing 0.1 mM histamine, they developed the ability to suppress the CTL response (Fig. 1, bar C) but cells preincubated in medium without histamine did not induce this suppressive activity (Fig. 1, bar D). Furthermore, culture of normal cells with histamine failed to induce this suppressive regulatory activity (data not shown). Thus, histamine can act as an inducer of "preactivated" suppressor cells, allowing them to express their potential under conditions in which they would not otherwise do so.

The histamine-induced suppressor cells from mice rendered tolerant with TNBS did not alter allo-CML responses (Table 1), indicating that the tolerization procedure did not result in the production of histamine-inducible suppressor cells with no antigen specificity.

Use of Histamine Agonists to Induce Suppressor Cells Reveals That Histamine Can Activate Opposing Regulatory Activities That Can Obscure Suppression. Although the results presented in Fig. 1 are both dramatic and clear-cut, this was

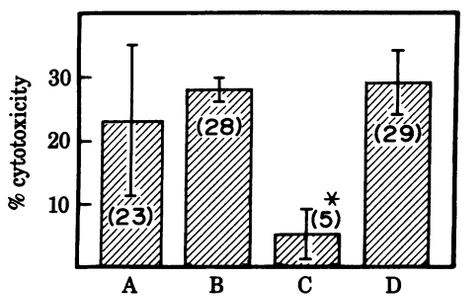

FIG. 1. Histamine treatment of cells from mice made tolerant with TNBS activates suppressors of anti-TNP CTL generation. Normal $B6AF_1$ spleen cells (5×10^6) were incubated with 5×10^5 TNP-labeled $B6AF_1$ stimulator cells in 5-day CML cultures. To these normal cells were added either nothing (control group, bar A) or 10×10^5 mitomycin C treated regulatory cells from mice that had received TNBS (bars B, C, and D). Before addition, these cells were preincubated with 0.1 mM histamine (bar C) or with medium (bar D) or were not preincubated (bar B). After 5 days, the cells were harvested and incubated with ^{51}Cr-labeled TNP target cells for 4 hr and percentage cytotoxicity was determined. Results are shown as mean ± SD; % cytotoxicity is shown in parentheses. *, Significantly different ($P < 0.01$) from controls.

Table 1. Antigen specificity of suppressor cells

Specificity of CTL generated in CML	% cytotoxicity	
	No added regulatory cells	With 10×10^5 regulatory cells
$B6AF_1$ anti-TNP-$B6AF_1$	23 ± 12	5 ± 4
$B6AF_1$ anti-BALB/c	28 ± 4	30 ± 5

CML cultures were generated with normal $B6AF_1$ spleen cells and either TNP-labeled $B6AF_1$ spleen cells or BALB/c spleen cell stimulators. Suppressor regulatory cells were from mice that had received TNBS intravenously and were incubated in 0.1 mM histamine for 60 min and then washed before addition to CML cultures. After 5 days, cells were harvested and incubated with ^{51}Cr-labeled TNP target cells and percentage cytotoxicity was determined. Results are shown as mean ± SD.

not a regular occurrence in these types of experiments. Dose–response experiments showed that amounts of regulatory cells varying from 1×10^5 to 20×10^5 sometimes produced comparable amounts of suppression. Although suppression was often seen after 30–60 min of preincubation with 0.01–0.1 mM histamine, in a significant number of experiments histamine failed to induce suppressive activity. In one experiment there was a paradoxical result: preincubation with 0.01 mM histamine gave significant suppression but preincubation with 0.1 or 1 mM did not.

These results led us to investigate the possibility that histamine might be inducing more than one type of regulatory cell. Histamine effects have been grouped into two types—H_1 and H_2 receptors (17)—according to the kind of receptor that accepts the histamine-induced signals. Histamine activates both kinds of receptors, but agonists have been synthesized that have relatively greater specificity for one or the other type. To see if we could demonstrate the activation of two types of opposing regulatory cells, we used two such agonists: PEA, which has greater specificity for H_1 receptors and acts with approximately equal potency to histamine (18); and dimaprit, which has greater H_2 specificity but is significantly less potent than histamine on an equimolar basis (19).

Preincubation with the H_1 agonist PEA induced no suppression in cells from TNBS-treated mice (Fig. 2, bar B). The H_2 agonist dimaprit, however, did induce suppression with greater efficiency than usually found with histamine (Fig. 2, bar C). However, when the regulatory cells were pretreated with both of the two synthetic agonists, the suppressive capacity of dimaprit was eliminated (Fig. 2, bar D). Thus, it seems that PEA is not inert in this system but is exerting an effect that nullifies suppressor cell induction by dimaprit. These results could explain (at least in part) the previously noted variability of histamine inducing cells from TNBS-treated mice in suppression of production of CTL. In some instances, histamine may appear to have no regulatory effect due to its activation of two opposing regulatory cell subsets.

Different Histamine Agonists That Induce Opposing Regulatory Activity Act on Separate Cells. To determine whether histamine agonists induced separate regulatory cells with opposing actions we performed a mixing experiment that tested whether cells made tolerant *in vivo* and then preincubated with PEA could block the suppressive effects of regulatory cells that had been preincubated with dimaprit. Preincubation of the regulatory cells with dimaprit led to a marked suppression (Fig. 3, bar D) and inclusion of PEA along with the dimaprit inhibited the suppression (Fig. 3, bar E). Furthermore, a separate aliquot of regulatory cells preincubated with PEA and added to the regulatory cells that had been preincubated only with dimaprit also inhibited the suppression (Fig. 3, bar F).

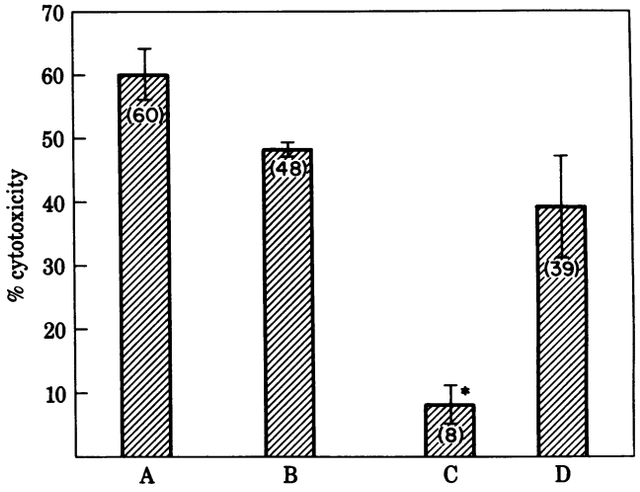

FIG. 2. Use of synthetic histamine agonists to induce regulatory activity in cells from mice made tolerant with TNBS. These cells were preincubated for 60 min at 37°C with synthetic histamine agonists (bar B, 0.1 mM PEA; bar C, 1 mM dimaprit; bar D, 0.1 mM PEA plus 1 mM dimaprit) and then were added to 5-day CML cultures of normal $B6AF_1$ responder cells and TNP-labeled stimulator cells (bars B, C, and D). Bar A represents cells from normal mice and preincubation without agonist. After 5 days, the cells were harvested and then incubated with ^{51}Cr-labeled TNP target cells for 4 hr and assayed for percentage cytotoxicity. Results are shown as mean ± SD; % cytotoxicity is shown in parentheses. *, Results significantly different ($P < 0.01$) from controls.

The simplest interpretation of our results is that immunization with TNBS stimulates a population of suppressor cells and that the activity of these cells can be influenced by histamine. However, their activity can be blocked by a second population of cells that express a different type of histamine "receptor" and are similarly histamine inducible.

PEA-Induced Contrasuppressor Cells Are Eliminated by Treatment with Anti-I-J Antibody Plus Complement. The ability of the cells preincubated with PEA to inhibit the suppressor activity of cells preincubated with dimaprit suggested that PEA activated cells in the recently described contrasuppressor circuit (2, 3). To test this we took advantage of the fact that the regulatory cells of the contrasuppressor circuit express a determinant controlled by the *I–J* subregion of the major histocompatibility complex. We took cells from mice that had received TNBS intravenously and incubated them for 1 hr with PEA, removed $I\text{-}J^+$ cells by treatment with anti-I-J antibody plus complement, and then added the cells to CML cultures in which anti-TNP CTL were being generated. Addition of cells preincubated with medium for 1 hr and then treated with complement before being added to CML cultures had a modest suppressive activity (Fig. 4, bar B) and treatment with anti-I-J antibody had little effect on their modest suppressive effect (Fig. 4, bar C). Preincubation with PEA reversed the suppressive effect and returned the CTL response to that of control cultures without regulatory cells added (Fig. 4, bar D). This is consistent with the previous finding that incubation with PEA can eliminate suppressor activity.

If this reversal of suppression were due to contrasuppressor cells that are $I\text{-}J^+$ and the suppressor cells were $I\text{-}J^-$ [as is true in other systems (2, 3)], then treatment with anti-I-J should restore suppressor activity in the PEA-treated cell population. Treatment with anti-I-J not only removed the cells that blocked the suppression seen with the cells incubated only with medium and treated with complement alone but also revealed the presence of active suppressor cells that inhibited the CTL response

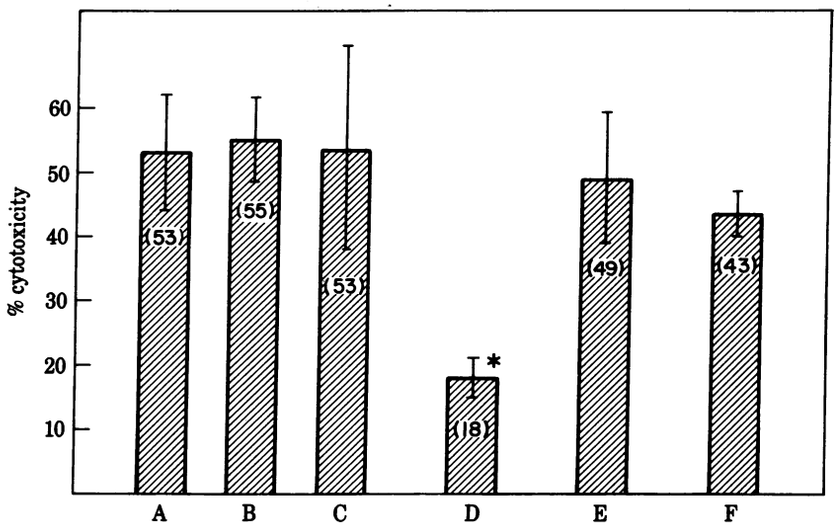

FIG. 3. Synthetic histamine agonists induce opposing regulatory activities in different aliquots of cells from mice made tolerant with TNBS. Regulatory cells were divided into two aliquots. Prior to being combined and then added or added alone to 5-day CML cultures of normal $B6AF_1$ responder cells and TNP-labeled stimulator cells, the aliquots were preincubated for 60 min at 37°C as follows: bar A, no regulatory cells added (control); bar B, aliquot 1 preincubated in medium; bar C, aliquot 2 preincubated with 0.1 mM PEA; bar D, aliquot 1 preincubated with 1 mM dimaprit; bar E, aliquot 1 preincubated with 1 mM dimaprit and with 0.1 mM PEA; bar F, aliquot 1 preincubated with 1 mM dimaprit and then combined with aliquot 2 that was preincubated with 0.1 mM PEA. After 5 days, the cells were harvested and incubated with ^{51}Cr-labeled TNP target cells for 4 hr, and then percentage cytotoxicity was determined. Results are shown as mean ± SD; % cytotoxicity is shown in parentheses. *, Significantly different ($P < 0.01$) from controls.

to 1/6th of control levels (Fig. 4, bar E). Thus, PEA activated a population of $I\text{-}J^+$ contrasuppressor cells. In fact, elimination of these cells revealed the presence of $I\text{-}J^-$ cells with even more potent suppressor activity than was found in cultures that did not have activated $I\text{-}J^+$ contrasuppressor cells.

DISCUSSION

These experiments were performed to determine if a model system for microenvironmental immune regulation could be formulated. The experiments were predicated on the idea that a cellular mediator, released at the site of immune reactions, could modulate immunity. Because we know that histamine is released at sites of delayed type hypersensitivity and can alter the strength and duration of this response (20), we used histamine induction of immunomodulatory cells as a model system—we generated suppressor cells to TNP by injecting TNBS and tested these putative suppressive cells for ability to regulate normal CML responses to TNP-modified self. In the absence of histamine, these *in vivo* immunized cells failed to produce significant regulation. However, they could be induced to do so by preincubation with histamine for <1 hr at concentrations (0.01–0.1 mM) that might be the levels found for such periods in local inflammatory sites.

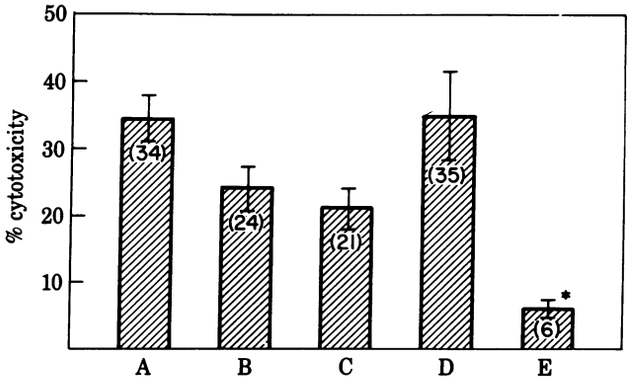

FIG. 4. Anti-I-J treatment of PEA-activated regulatory cells from TNBS-treated mice removes contrasuppressor activity. Cells from mice made tolerant by intravenous injection of TNBS (bars B, C, D, and E) were preincubated for 60 min at 37°C and then, before being added to 5-day CML cultures of normal $B6AF_1$ responder cells and TNP-labeled stimulator cells, were pretreated as follows: bar A, no regulatory cells added (control); bar B, no preincubation, then pretreatment with complement only; bar C, anti-I-J and complement pretreatment only; bar D, preincubation with 0.1 mM PEA only; bar E, preincubation with 0.1 mM PEA, then pretreatment with anti-I-J and complement. After 5 days, cells were harvested and incubated with ^{51}Cr-labeled TNP target cells for 4 hr and then percentage cytotoxicity was determined. Results are shown as mean ± SEM; % cytotoxicity is shown in parentheses. *, Significantly different ($P < 0.01$) from controls.

Although often dramatic, the ability of histamine to induce suppressor cells was variable and gave nonstoichiometric, and sometimes even paradoxical, dose–response curves. To try to understand this variability and unusual dose–response, we made the assumption that histamine could induce both suppressor and contrasuppressor cell (2, 3) activities and that these mutually opposing effects of the same inducer molecule were responsible for the variability and other idiosyncrasies found. We tested this idea by using two different histamine agonists to induce immunoregulatory effects. The H_2 agonist dimaprit was a more efficient inducer of suppressor cells than was histamine whereas the H_1 agonist PEA had a greater effect on contrasuppressor cells. Thus, regulatory cells preincubated with PEA could block the suppressive effect of regulatory cells preincubated with dimaprit. The PEA-activated cells resembled contrasuppressor cells described in other systems because blockage of suppression was removed by anti-I-J treatment (2, 3). In fact, potent $I\text{-}J^-$ suppressor activity was revealed by elimination of the PEA-activated $I\text{-}J^+$ cells.

There could be at least two reasons for the concomitant augmentation of suppression in the presence of PEA-induced contrasuppression. (*i*) PEA is predominately an H_1 agonist but does have weaker H_2 agonist effects (18). Preincubation of regulatory cells with PEA thus may induce both $I\text{-}J^+$ contrasuppressors and

$I\text{-}J^-$ suppressors, but the former predominate. (*ii*) Contrasuppressor cells act by making the targets of suppressor cells resistant to suppression (21). Thus, we often find that the presence of activated contrasuppressor cells leads to a concomitant increase in latent suppressor cells, probably because more suppression is required to achieve immunological homeostasis when the suppressor cell's target cell becomes more resistant to the signals of the suppressor cells. Thus, an increase in suppressor activity of $I\text{-}J^-$ cells may arise directly due to modest H_2 agonist effects of PEA or may arise indirectly with activation of $I\text{-}J^+$ contrasuppressors, as a requirement to achieve a homeostatic balance. In either case, PEA activated a potent population of $I\text{-}J^+$ cells that prevented expression of potent suppressor cell activity.

Taken together, these results support the contention that release of local mediators such as histamine at sites of immune reactions could regulate the intensity of the reaction at the site. This type of system in principle would be an efficient one for turning local immune responses off or on, depending upon need (which would be signaled by the release of various types of mediators or inducers at these local sites) and preferential local recruitment of cells with appropriate receptors for these inducing signals. Previous observations have suggested that histamine plays a role as a mediator of immunoregulation; our results differ from previous findings in three ways: (*i*) nearly all studies have found histamine to be suppressive (7), whereas we observed a bidirectional potential for immunomodulation by histamine; (*ii*) except for our demonstration of histamine activation of regulatory cells, and the findings of Rocklin *et al.* (22) that histamine can induce production of a nonspecific suppressive factor, other findings with histamine may have been due to a direct suppressive action on immune cells, perhaps via increases in cyclic AMP (4, 7); and (*iii*) our findings were produced by a brief exposure to histamine, whereas all other effects have required the continuous presence of histamine, a situation unlikely to occur *in vivo*. Therefore, the potential for bidirectional effects and induction of preactivated antigen-specific regulatory cells after a brief exposure make histamine an ideal candidate for a mediator of local immune regulation *in vivo*.

It is important to emphasize that we have not shown that histamine or any other agonist we have used works on histamine-specific receptors. At least in theory, all of the effects described could be due to the charged nature of histamine or the specific agonists or to other nonspecific properties they might have. Furthermore, one cannot ascribe a specific role or immunoregulatory productive role to H_2 versus H_1 agonists from these data. Considerably more pharmacological data are needed before such conclusions can be drawn. However, recent binding studies indicate that H_1 receptors may be present on T cells (23) and our finding that PEA works as an agonist may be taken as evidence that these are functional receptors. We did not use combinations of histamine and receptor-specific histamine antagonists because in preliminary experiments we found that the antihistaminics had actions of their own in our system, as has been found in other systems (24). Although we have tended to interpret our results with dimaprit and PEA in terms of these drugs being agonists, we have not ruled out the possibility that in some circumstances they might act as antagonists by mechanisms such as tachyphylaxis.

These caveats do not alter the basic message. Histamine at roughly physiological concentrations and duration of exposure can act as an immunoregulatory inducing agent, independently of the receptor it works on. Furthermore, the data show that histamine-like agents can have more than one immunoregulatory effect and, under some circumstances, these two effects can cancel one another out. It is important to stress that the two opposing effects induced by histamine-like drugs can occur by working on separate cells and are not necessarily a cancellation of one cellular signal by another on the same cell.

In summation, our results indicate that the functional activity of suppressor and contrasuppressor cells can be modulated by local mediators released during the course of an immune reaction. This type of feedback activity can increase the efficiency of immunomodulation by confining it to areas where an immune response is taking place.

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1. Cantor, H. & Gershon, R. K. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2058–2064.
2. Gershon, R. K., Eardley, D. D., Durum, S., Green, D. R., Shen, F. W., Yamauchi, K., Cantor, H. & Murphy, D. B. (1981) *J. Exp. Med.* **153**, 1533–1546.
3. Yamauchi, K., Green, D. R., Eardley, D. D., Murphy, D. B. & Gershon, R. K. (1981) *J. Exp. Med.* **153**, 1547–1561.
4. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y. & Shearer, G. M. (1974) *Science* **184**, 19–28.
5. Rocklin, R. E. (1976) *J. Clin. Invest.* **56**, 1051–1058.
6. Rocklin, R. E., Greineder, D., Littman, B. H. & Melmon, K. L. (1978) *Cell. Immunol.* **37**, 162–173.
7. Melmon, K. L., Rocklin, R. E. & Rosenkranz, R. P. (1981) *Am. J. Med.* **71**, 100–106.
8. Askenase, P. W., Bursztajn, S., Gershon, M. D. & Gershon, R. K. (1980) *J. Exp. Med.* **152**, 1358–1374.
9. Schwartz, A., Askenase, P. W. & Gershon, R. K. (1977) *J. Immunol.* **118**, 159–165.
10. Zembala, M. & Asherson, G. L. (1973) *Nature (London)* **244**, 227–228.
11. Fujiwara, H., Levy, F. B., Shearer, G. M. & Terry, W. D. (1979) *J. Immunol.* **123**, 423–425.
12. Finberg, R., Greene, M. I., Benacerraf, B. & Burakoff, S. J. (1979) *J. Immunol.* **123**, 1205–1209.
13. Finberg, R., Burakoff, S. J., Benacerraf, B. & Greene, M. I. (1979) *J. Immunol.* **123**, 1210–1214.
14. Gey, G. O. & Gey, M. K. (1936) *Am. J. Cancer* **27**, 45–76.
15. Schwartz, A., Askenase, P. W. & Gershon, R. K. (1980) *Immunopharmacology* **2**, 179–190.
16. Schwartz, A., Sutton, S. L., Askenase, P. W. & Gershon, R. K. (1981) *Cell. Immunol.* **60**, 426–439.
17. Black, J. W., Duncan, W. A. M., Durant, G. J., Ganellin, C. R. & Parsons, M. E. (1972) *Nature (London)* **236**, 385–390.
18. Durant, G. J., Ganellin, C. R. & Parsons, M. E. (1975) *J. Med. Chem.* **18**, 905–909.
19. Parsons, M. E., Owens, D. A. A., Durant, G. J. & Ganellin, C. R. (1977) *Agents Actions* **7**, 31–37.
20. Askenase, P. W., Schwartz, A., Siegel, J. & Gershon, R. K. (1981) *Int. Arch. Allergy Appl. Immunol.* Suppl. 1, **66**, 225–233.
21. Green, D. R., Eardley, D. D., Kimura, A., Murphy, D. B., Yamauchi, K. & Gershon, R. K. (1981) *Eur. J. Immunol.* **11**, 973–980.
22. Rocklin, R. E., Greineder, D. K. & Melmon, K. L. (1979) *Cell. Immunol.* **44**, 404–415.
23. Beer, D. J., Osband, M. E., McCaffrey, R. P., Soter, N. A. & Rocklin, R. E. (1982) *N. Engl. J. Med.* **306**, 454–458.
24. Schwartz, A., Sutton, S. L. & Gershon, R. K. (1982) *J. Exp. Med.* **155**, 783–796.