

# The Regulatory Effect of Histamine on the *in Vitro* Synthesis of IgE

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The effect of histamine and its H<sub>1</sub> and H<sub>2</sub> antagonists, chlorpheniramine and cimetidine, on the *in vitro*, PWM-induced, synthesis of IgG and IgE was studied. Histamine had no effect, and cimetidine had a slight inhibitory action. In contrast, chlorpheniramine induced marked suppression of both IgE and IgG synthesis. This effect could not be attributed to drug-induced cytotoxicity. These results suggest that the modulatory effect of histamine on antibody production involves predominantly H<sub>1</sub> receptors.

**Key words:** histamine; histamine-antagonists; IgE synthesis.

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Histamine has been shown to influence a variety of immunologic phenomena. Thus, far, its effects on lymphocyte function have been studied in great detail in T-cell systems. Histamine inhibits T-cell cytotoxicity, T-cell proliferation to mitogens and antigens, and lymphokine production. Most of these effects involve the activation of histamine type 2 (H<sub>2</sub>) receptors on suppressor T-cells which then results in the production of a soluble suppressor factor (histamine-induced suppressor factor or HSF) (1, 5, 6, 7, 10).

The effect of histamine on B-cell function, i.e. antibody production, has been less well studied. Several investigators have shown that histamine has a suppressive effect on immunoglobulin production. These studies performed in both the murine and the human systems, were limited to the synthesis of IgM and IgA (1, 2, 3, 9). In the present study we wanted to extend these studies to the IgE system, namely to evaluate the modulatory effect of histamine and its H<sub>1</sub> and H<sub>2</sub> antagonists on the *in vitro* synthesis.

## PATIENTS AND METHODS

The study group comprised eight healthy non-atopic individuals. Their mean serum IgE was 30 IU/ml.

Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradients. Following washing, the cells were incubated in RPMI-1640 (GIBCO) supplemented with 20 % fetal calf serum (FCS: Flow Laboratories), 2 mM L-glutamine, 100 u/ml streptomycin, and 2.5 mM N-2-hydroxyethylpiperazine-N-2 ethanesulfonic acid (HEPES) buffer. Cultures, 2 ml each, were performed in 16 mm flat-bottomed tissue culture wells (Linbro 24 Wells, Flow Laboratories) with a cell concentration of  $1.0 \times 10^6$ /ml in the presence of PWM (GIBCO), final dilution 1:100. Cell viability was tested by the trypan blue method. Cultures were run in duplicate and harvested on day 7. Supernatants were collected and each tested for both IgE and IgG.

Cultures were performed in the absence or presence of each of the following drugs: hist-

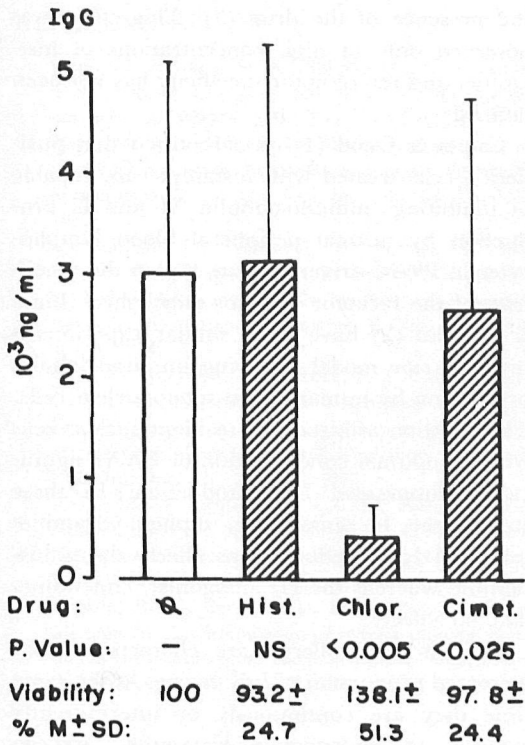


Fig. 1. Effect of histamine, chlorpheniramine and cimetidine on IgG synthesis, and cell-viability.

amine (Sigma),  $H_1$  antagonist-chlorpheniramine and  $H_2$  antagonist-cimetidine (both kindly donated by SK&F). The pharmacologic concentrations of these agents range from  $10^{-5}$  to  $10^{-4}$  M. In preliminary experiments  $10^{-4}$ – $10^{-6}$  concentrations were used; however, the following results are based on the optimal concentration of  $10^{-4}$ .

IgE was determined by a radioimmunoassay as described by Saxon & Stevens (8). Anti IgE antibody was obtained from Meloy (Springfield, VA). Standard curves were established by IgE obtained from Pharmacia (PRIST Kits). IgE levels as low as 50 pg/ml could be detected by this assay. IgM, IgG or light chains were not detected by this assay even when used in mg quantities. The binding of  $^{125}$ I anti-human IgE to the wells during the second incubation could be inhibited by purified human IgE but not by IgG, IgM or light chains. IgG was determined by radioimmunoassay, as described by Ohta et al. (4).

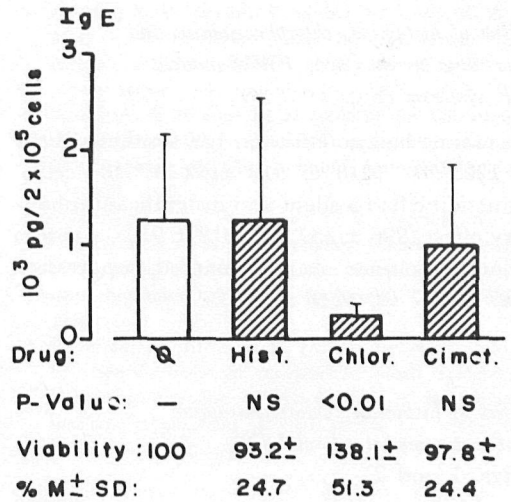


Fig. 2. Effect of histamine, chlorpheniramine and cimetidine on IgE synthesis.

*Statistical analysis.* Immunoglobulin concentrations have a normal distribution once converted to their corresponding log-values. For this reason, the geometric means of both IgG and IgE were used for the statistical analysis, performed by the paired t-test.

## RESULTS

### *In vitro synthesis of IgG and IgE in unstimulated cultures*

Immunoglobulin synthesis in the absence of PWM was minimal:  $211 \pm 92$  ng/ml for IgG and  $249 \pm 145$  pg/ $2 \times 10^5$  cells for IgE. Therefore, the regulatory effect of the various drugs on Ig synthesis was evaluated only in PWM-stimulated cultures.

### *Effect of histamine, chlorpheniramine and cimetidine on in vitro, PWM-induced IgG synthesis (Fig. 1)*

Histamine had no effect on IgG synthesis ( $3138 \pm 2141$  vs.  $3013 \pm 2164$  ng/ml). Cimetidine had a slight inhibitory effect ( $2668 \pm 2108$  vs.  $3013 \pm 2164$ ), whereas chlorpheniramine caused marked suppression ( $443 \pm 295$  vs.  $3013 \pm 2164$ ).

*Effect of histamine, chlorpheniramine and cimetidine on in vitro, PWM-induced IgE synthesis (Fig. 2)*

Histamine had no effect on IgE synthesis ( $1251 \pm 1265$  vs.  $1218 \pm 919$  pg/ $2 \times 10^5$  cells). Cimetidine had a slight and insignificant inhibitory effect ( $996 \pm 837$  vs.  $1218 \pm 919$ ), whereas chlorpheniramine caused marked suppression ( $247 \pm 137$  vs.  $1218 \pm 919$ ).

*Effect of histamine, chlorpheniramine and histamine on cell viability (Figs. 1 and 2)*

Recovery of viable cells on day 7 of culture was determined to evaluate possible cytotoxic effect of the added drugs. Histamine and cimetidine had no effect on viability ( $93.2 \pm 24.7$  and  $97.8 \pm 24.4\%$ ; in contrast, the presence of chlorpheniramine resulted in increased proliferation ( $138.1 \pm 51.3\%$ ).

## DISCUSSION

Histamine has a diverse modulatory action on the immune system (5).

The effects on T-cell function have been studied in detail and some are well established. Thus, for example, suppressor, OKT-8 cells seem to have histamine specific receptors. These receptors can be activated by histamine and  $H_2$  agonists and blocked by  $H_2$  antagonists, such as cimetidine. This activation results in the production of a soluble suppressor factor (HSF) which inhibits T-cell proliferation and lymphokine production (1, 5, 6, 7, 10).

In contrast, only few investigators have studied the regulation of B-cell response by histamine and its analogues, and the results obtained are still somewhat controversial. Shearer et al. (9) have shown that plaque-forming-cell number may be increased if murine splenic lymphocytes are first chromatographed on columns containing insolubilized histamine. Furthermore, histamine may inhibit plaque formation when murine spleen cells are cultured in

the presence of the drug (3). This effect was observed only at high concentrations of histamine, and the receptor specificity has not been defined.

Gupta & Good (1) have reported that purified T-cells treated with histamine are capable of inhibiting immunoglobulin M and G production by normal peripheral blood lymphocytes in PWM-driven system. Again the specificity of the receptor was not established. Lima & Rocklin (2) have done similar experiments in an *in vitro* model involving immunoglobulin production by human blood mononuclear cells. The addition of histamine to mononuclear cells with an optimal concentration of PWM significantly suppressed IgG production. In these studies the  $H_1$  antagonist, diphenhydramine, inhibited IgG production as effectively as histamine, whereas the  $H_2$  antagonist, cimetidine, had no effect.

Patients with allergy are characterized by increased production of IgE *in vivo*. At the same time they are continuously or intermittently exposed to endogenous histamine, and to exogenously administered histamine antagonists usually of the  $H_1$  type. In view of the known modulatory effects of these agents on the immune system, we wanted in the present study to evaluate the potential influence of these drugs on IgE synthesis. In our *in vitro* model of PWM-driven immunoglobulin E and G synthesis, we found that whereas histamine and cimetidine had minimal or no effect, the  $H_1$  antagonist chlorpheniramine had a striking suppressive influence on both IgE and IgG production. This effect was not due to drug-induced cytotoxicity.

We consistently observed an increase in cell viability in cultures supplemented with chlorpheniramine.

Our finding, together with those of Lima & Rocklin (2) suggest that the modulatory effect of histamine on antibody production involves predominantly  $H_1$  receptors. This contrasts with the effect on T-cell function, which is primarily mediated by  $H_2$  receptors.

The therapeutic implications of the observed effect of  $H_1$  blocker on IgE synthesis should be evaluated in additional *in vitro* and *in vivo* studies.

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