Histamine release reaction of basophils in chronic granulocytic leukaemia

Induction by heterologous anti-immunoglobulin E, concanavaline A, and phytohaemagglutinin; effect of heavy water

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SUMMARY Basophils possess membrane bound IgE molecules, and immunological activation leads to a secretory process with cell degranulation and histamine release. Heterologous anti IgE, concanavaline A, and phytohaemagglutinin are potent non-cytotoxic releasing agents. They operate by a mechanism similar to that of immunological activation. Heavy water is not a histamine releasing inducer but it increases histamine release of the cells. We studied the histamine release reaction of leukaemic basophils in 10 patients and found a physiological response such as that previously reported with normal human basophils.

The blood picture of chronic granulocytic leukaemia (CGL) shows a progressive accumulation of granulocytes of varying maturity but without gross morphological change. Although these may be minor non-specific abnormalities, circulating mature basophils, as the majority of mature neutrophils, are morphologically indistinguishable from normal cells. Since granulocyte function has been reported to be altered in CGL (Olofsson *et al.*, 1976), the present study was designed to evaluate the function of CGL basophils by the histamine release reaction, as previously performed in normal human and other mammalian leucocytes (Hastie, 1971; Ishizaka *et al.*, 1972; Siraganian and Siraganian, 1975). Our results seem to demonstrate the existence of a normally induced histamine release reaction in CGL basophils.

Material and methods

Peripheral leucocytes were obtained from 10 patients with CGL. They had no previously known allergies. Release of histamine was performed using a slight modification (human serum was not used in the reaction medium) of the technique described by May *et al.* (1970). Leucocytes were isolated from fresh

heparinised venous blood by dextran sedimentation. After washing in tris albumin buffer (Tris A), the cells were incubated for one hour at 37°C in Tris A with calcium and magnesium (Tris ACM:NaCl 120 mmol, KCl 5 mmol, Tris 25 mmol, human serum albumin 0.03%, Ca⁺⁺ 1 mmol, Mg⁺⁺ 1 mmol), adjusted to pH 7.4, and with an appropriate amount of anti IgE,¹ concanavaline A² (Con A), and phytohaemagglutinin³ (PHA).

Cells were subsequently removed by centrifugation, and the supernatant was assayed for histamine by the fluorimetric technique of Shore *et al.* (1959).

The residual histamine content of the cells was determined after extraction by boiling for four minutes. Release of histamine was corrected for spontaneous release from control cells, and the result was expressed as net per cent of histamine release. In a second stage, heavy water⁴ (D₂O) partially replaced H₂O in the medium (78%). All the media were adjusted to the same osmolarity by the

¹Sheep anti human IgE (Pharmacia, Uppsala, Sweden).

²Concanavaline A (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

³Bacto Phytohemagglutinin P (Difco Laboratories, Detroit, Michigan, USA).

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Fig. 1 Anti IgE induced histamine release from leucocytes of 10 patients (anti IgE in serial dilutions from a sample containing 0.2 to 0.5 mg/ml of sheep antihuman IgE antibody). The dose-response curves show for each patient a maximal HR (which characterises the cellular reactivity) for an optimal anti IgE concentration (which characterises the cellular sensitivity). Decrease of HR is observed with excess of anti IgE.

addition of sodium chloride. All experiments were performed in duplicate, the average difference between duplicate measurements being less than 5%.

Results

ANTI IgE, CON A, AND PHA INDUCED HISTAMINE RELEASE

The leucocyte histamine release (HR) of 10 patients with increasing concentrations of the three inducers assayed is shown in Figs 1 to 3.

The responses of different individual leucocytes indicate a wide variation but, as is clearly shown with anti IgE, there is an optimal concentration giving maximum HR. This reaction is inhibited with supra-optimal doses. The optimal concentration of inducer giving maximum HR is defined as the cellular sensitivity (Table 1).

All 10 patients responded to anti IgE and Con A while only six responded to PHA. Moreover, the best HR was obtained with anti IgE, and the worst with PHA (Table 2). The maximum percentage of HR is defined as the cellular reactivity.

We observed no significant correlation in cellular reactivities and sensitivities ($r = -0.4$, $P > 0.10$) with the three inducers.

EFFECT OF D₂O ON INDUCED HR

In nine out of the 10 patients, the addition of D₂O to the medium resulted in a marked increase of the induced HR (Table 2). There was no change in the cellular sensitivity, the HR curves only being trans-

Fig. 2 Con A induced histamine release (Con A in µg/ml). The dose-response curves of the 10 patients' leucocytes show a pattern similar to that obtained with anti IgE. Nevertheless maximal HR is less important and the curves are more widespread.

Fig. 3 PHA induced histamine release (PHA in $\mu\text{g/ml}$). Compared to anti IgE and Con A, PHA is a poor inducer for HR. Even with high (non-cytotoxic) PHA concentrations, four out of 10 patients' leucocytes (patients 1, 2, 3, 10) do not release any histamine. Except for patient 6, maximal HR does not exceed 10%.

Table 1 Cellular sensitivity (concentration of the inducer for maximum HR)

Histamine release inducer	Range	Median
Anti IgE (dilution from initial sample)	1×10^{-8} to 1×10^{-3}	1×10^{-4}
Con A ($\mu\text{g/ml}$)	1 to 10^3	50
PHA ($\mu\text{g/ml}$)	10^2 to 10^3	5×10^2

Table 2 Cellular reactivity with and without D_2O (maximum % of HR)

No. of patients	Anti IgE	Anti IgE + D_2O	Con A	Con A + D_2O	PHA	PHA + D_2O
1	41	100	24	93	0	52
2	50	91	15	81	0	0
3	25	99	4	94	0	33
4	27	37	10	49	4	30
5	36	91	6	84	4	31
6	38	86	22	100	11	70
7	33	100	3	88	2	61
8	64	93	17	100	2	72
9	41	100	4	47	8	61
10	19	84	29	95	0	62
Mean $\pm$ SEM	37 ± 4	88 ± 6	13 ± 3	83 ± 6	3 ± 1	47 ± 7

lated higher. This increase of HR was observed also in three patients who did not release histamine with PHA alone. Moreover, five patients, not included in this study, who did not show HR with anti IgE, Con A, or PHA, showed a significant HR with these three inducers in the presence of D_2O .

The increase of the mean HR with D_2O is +50% for anti IgE, +80% for Con A, and +44% for PHA, the differences being highly significant ($P < 0.001$).

Discussion

After appropriate stimulation, human basophilic leucocytes release several pharmacological mediators. One of these mediators, histamine, is preformed within the cell, confined to specific cytoplasmic granules (Ackerman, 1963; Sampson and Archer, 1967).

It has been shown that normal basophils possess membrane bound IgE molecules and that antigen induced HR from basophils of atopic individuals depends on the bridging of two specific membrane bound IgE molecules (Hastie, 1971; Ishizaka *et al.*, 1972). This HR is not accompanied by cytotoxicity but is related to the degranulation of the cell, depending on a secretory process (Hastie, 1971). Heterologous anti IgE can also activate the basophils (Ishizaka *et al.*, 1970) in an analogous fashion.

Lectins (Con A, PHA) are also potent non-cytotoxic histamine releasing agents (Siraganian and Siraganian, 1975). By a mechanism similar to that of the immunological activation of basophils, they bind to carbohydrate groups of the Fc fragment of two IgE molecules, bridging them together (Keller, 1973; Siraganian and Siraganian, 1974, 1975).

Heavy water is not a histamine releasing agent for human basophils but it increases HR by its stabilising effect on the microtubular system of the cell (Gillespie and Lichtenstein, 1972) whatever is the histamine releasing inducer.

Our study indicates that leukaemic human basophils, as normal human basophils, can release their histamine content after activation.

Previous experiments (Lewis *et al.*, 1975; Siraganian *et al.*, 1975; Dvorak *et al.*, 1976) on leukaemic basophils gave contradictory results.

Siraganian *et al.* (1975), using ionophore A-23187 as releasing reagent on rat basophil leukaemia cells, did not obtain IgE mediated HR, the cells responding only to high cytotoxic doses. Lewis *et al.* (1975) obtained histamine release of basophils in a CGL patient with calcium ionophore but not with rabbit anti-human IgE or compound 48/80.

Dvorak *et al.* (1976) obtained HR from leukaemic basophils by an ionophore but not by antibodies directed at IgE. However, the above-mentioned reports are based on only one patient, and D_2O was not used to potentiate HR. It has been shown that certain normal subjects do not release histamine from their basophils (Ishizaka *et al.*, 1973) as did

our five patients who showed HR only after the addition of D₂O.

Our study clearly indicates that CGL basophils can bind anti IgE, Con A, and PHA, resulting in an activation of the cells and HR. This reaction occurs as in physiological conditions with a wide variation in the response of different individual basophils but with a dose dependency curve characterised by an increasing response with the concentration of the inducing agent, a maximum of HR, and an inhibition of the reaction by an excess amount of inducing agent (Siraganian and Siraganian, 1974, 1975). Moreover, heavy water potentiates HR from leukaemic basophils. This effect is best shown when testing patients whose leucocytes release little or no histamine under normal conditions. This indicates that, even though histamine is not released, CGL basophils have actually been activated by contact with anti IgE or lectins.

The histamine releasing function of the leukaemic basophil is thereby normal. The cell possesses IgE surface receptors, allowing a physiological and immunological activation, and a normal microtubular system, which is necessary for the actual secretion of histamine (Gillespie and Lichtenstein, 1972).

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