

Spontaneous histamine release from washed leukocytes and whole blood in atopic and non-atopic individuals

U. WAHN, M. ERNSTING & J. PETERSON

University Children's Hospital, Freie Universität Berlin, W. Germany

The ability of basophils from patients with allergic rhinitis, extrinsic asthma, chronic idiopathic urticaria and atopic dermatitis to release histamine spontaneously *in vitro* was studied. Spontaneous release in the presence and absence of 30% D₂O was investigated from both washed leukocytes and whole blood. Compared with controls histamine release (HR) from washed leukocytes was significantly enhanced in allergic rhinitis patients only, whereas in the other groups only a certain percentage of patients was found to have high spontaneous HR. In whole blood experiments HR in all groups was within the normal range. Our data indicate that enhanced spontaneous mediator release from washed basophils *in vitro* does not necessarily prove this mechanism to be of pathophysiological relevance *in vivo*.

Key words: atopy; basophils; histamine release (HR); releasability.

Accepted for publication 24 August 1989

Washed basophils from asthmatic and some groups of atopic subjects compared with non-atopic have been demonstrated to show differences in their ability to secrete histamine spontaneously (15). Since IgE-mediated mechanisms do not explain all cases of asthma, particularly intrinsic asthma, as well as atopic dermatitis or many forms of urticaria, it has been postulated that abnormal mediator release as a result of an increased releasability might reflect an intrinsic cellular component of some of these diseases (5).

A number of standard stimuli besides anti-IgE have been investigated for releasability in various pathophysiological conditions (2, 3, 7, 9, 10, 11, 12, 15). A summary of these studies, which have all been using washed leukocytes, is given in Table 1. Some of these stimuli, like D₂O and the calcium ionophore 23187, have been shown to induce signifi-

cantly more mediator release from washed basophils in patients with atopic dermatitis than in controls. In asthmatics D₂O-induced histamine release (HR) has been found to be enhanced (15), while C5_a and F-met-peptide-induced release is reported to be diminished in this group (3). Hyperosmolar mannitol and calcium ionophore as stimuli have been found equally potent in both asthmatics and controls (3).

Mediator release *in vitro* from washed basophils is an excellent experimental model for the study of human allergy. However, since whole blood appears to resemble physiological *in vivo* conditions more closely than washed cells, we performed the following study in order to demonstrate whether "spontaneous" HR *in vitro* occurs in whole blood as it does from washed leukocytes in the absence and presence of D₂O.

Table 1

The release of histamine from washed leukocytes of patients with urticaria, asthma and atopic dermatitis, induced by standard stimuli in comparison with controls

	H ₂ O	D ₂ O	Anti-IgE	C5a	f-Metpept.	Mannitol	Ca ⁺⁺ Ionophore
Chronic idiopathic urticaria			-				
Asthma		+	NS	-	-	NS	NS
Atopic dermatitis	+	+	+		(+)		+

+ = enhanced release compared with controls; - = reduced release compared with controls; NS = no significant differences compared with controls.

PATIENTS AND METHODS

The study comprised 15 patients (age-range 12 to 51 years) with allergic rhinitis (AR) by history and skin tests, 42 patients (age-range 2 to 52 years) with atopic dermatitis (AD), according to the criteria of Hanifin & Rajka (4), 14 with asthma (age-range 2 to 40 years) in association with atopy (at least one positive skin test to any environmental allergen), 19 (age-range 3 to 60 years) with chronic idiopathic urticaria (CIU) and 22 non-allergenic controls (age-range 4 to 50 years, negative history for allergies, negative skin test). Therapy with systemic steroids, theophylline, anti-histamines and ketotifen was discontinued for a period of at least 3 weeks prior to the investigation.

Venous blood was drawn from all patients, 10 ml for dextran sedimentation in order to obtain washed leukocytes, and 10 ml were heparinized and used for whole blood experiments.

Reagents

A Pipes-buffered (pH 7.4) medium was used containing NaCl 110 mM, KCl 5 mM, NaOH 40 mM, Pipes 25 mM, and 0.03 % human serum albumin (Pipes A). To this medium, 2 mM Ca⁺⁺ and 0.5 mM Mg⁺⁺ were added to prepare Pipes ACM used for diluting antigens and cells for the HR reaction. Stock EDTA solution was 0.1 M, pH 7.2. Media containing D₂O were prepared by

substituting D₂O for water in Pipes ACM, so that D₂O concentrations were 60 %.

Cell preparations and histamine release

20 ml of blood were collected in plastic syringes, 10 ml were anticoagulated with 0.01 M EDTA, pH 7.2 and sedimented at room temperature for 60 to 75 min with 2.5 ml dextran (clinical dextran 6% in 0.85 NaCl, to which 3 % glucose was added) per 10 ml of blood. The upper layer, containing leukocytes, platelets, and plasma, was transferred by pipetting to another plastic tube and then centrifuging at 300 × g for 8 min at 4°C. The pelleted cells were suspended in Pipes A buffer containing 4 mM EDTA and centrifuged at 300 × g for 8 min at 4°C. The cells were then washed in Pipes A. After centrifugation, the cells were suspended in Pipes ACM buffer. The other 10 ml were heparinized and kept at 4°C until used for HR experiments.

HR reactions were carried out in 12 × 75 mm plastic tubes. In washed leukocyte experiments 0.25 ml of either Pipes ACM-H₂O or Pipes ACM-D₂O were added to the tubes in an ice bath, 0.25 ml of the washed leukocytes was then added to all the tubes, and racks were transferred to a 37°C bath. The racks were shaken every 10 min to resuspend the cells. In each experiment, perchloric acid at a final concentration of 3 % was added to some samples to obtain the total histamine content of the cells. At the end of 60 min, the tubes were transferred to an ice bath and centri-

fuged at 1500 rpm ($650 \times g$) for 15 min at 4°C .

In whole blood experiments 0.25 ml of heparinized blood were used instead of washed cells.

In all tests experimental tubes were in quadruplicate. Histamine analysis was performed by an automated fluorometric technique as described by Siraganian (14).

Statistical methods

Spontaneous HR in relation to controls was analysed by Wilcoxon's signed rank sum test.

RESULTS

Spontaneous HR in the N group in an H_2O buffer ranged from 0 to 12 % after 1 h, and was significantly ($P < 0.01$) enhanced after 5 h up to 3–17 % (data not shown). In a buffer containing 30 % D_2O spontaneous release from washed cells ranged from 2–12 % after 1 h of incubation time and from 3 to 16 % after 5 h.

Parallel experiments in whole blood with the same incubation periods and D_2O concentra-

tions showed neither a significant increase in spontaneous release with increasing time nor in the presence of D_2O .

Allergic rhinitis

Compared with controls, AR patients as a group showed a higher spontaneous release from washed leukocytes, in the absence of D_2O ($P < 0.025$) (Fig. 1). At 30 % D_2O (Fig. 2) there was a tendency for higher HR which did not reach statistical significance. In contrast, release in whole blood in this group was not significantly different from controls.

Extrinsic asthma

In the group of asthmatic patients spontaneous HR exceeded 20 % only once. Among asthmatic patients individuals with concomitant atopic eczema tended to show higher spontaneous HR, which did not reach statistical significance. HR from whole blood was within the range of normal controls, both in the absence and presence of D_2O .

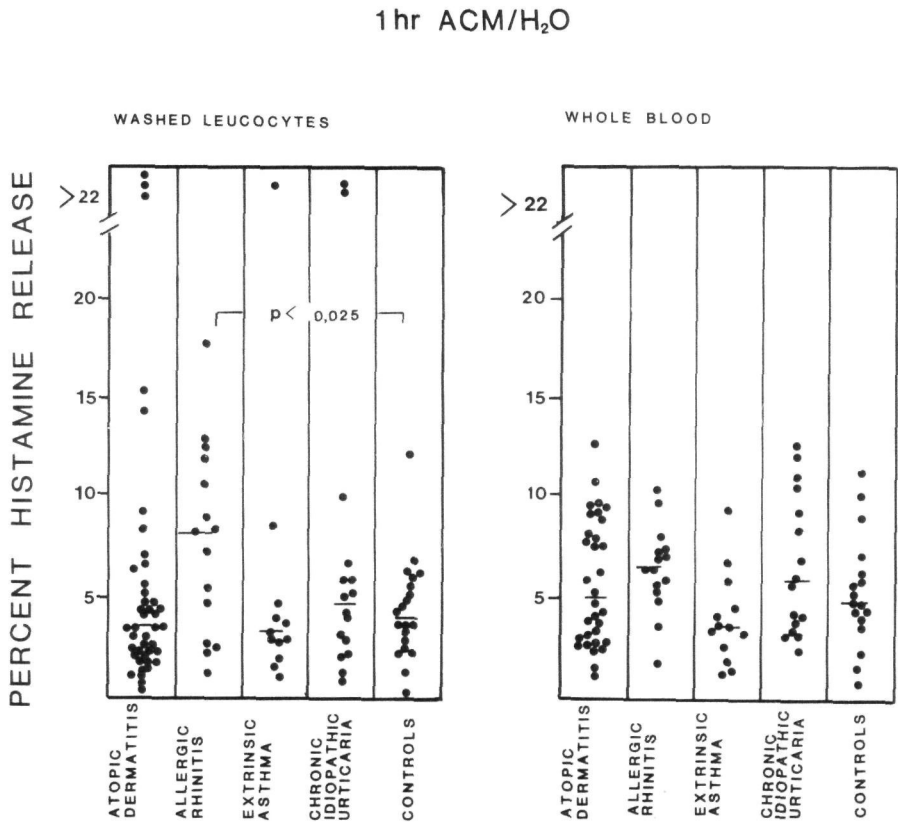


Fig. 1. Spontaneous histamine release *in vitro* from washed leukocytes and whole blood after 1 h of incubation in a Pipes buffered medium without D_2O .

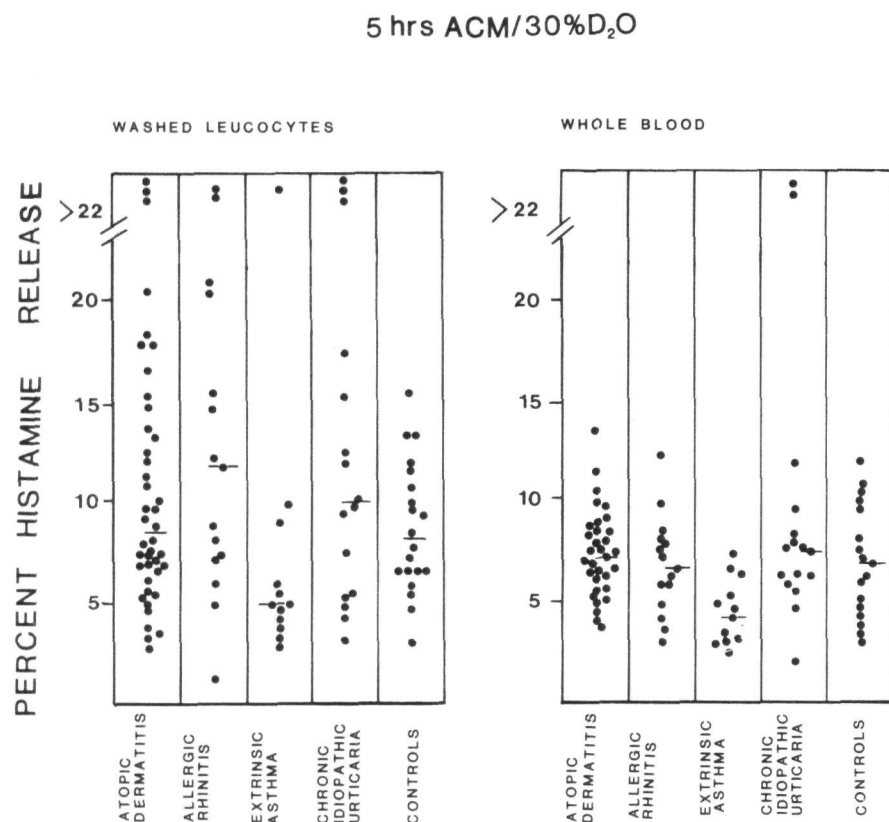


Fig. 2. Histamine release *in vitro* from washed leukocytes and whole blood after 5 h incubation in a Pipes buffered medium containing 30 % D₂O.

Chronic idiopathic urticaria

In the IU group only two patients in an H₂O buffer and three in 30 % D₂O showed more than 20 % HR from washed leukocytes. After 5 h incubation at 30 % D₂O these two were the only individuals who gave repeatedly more than 15 % HR even in whole blood in a D₂O buffered medium. Both patients had a positive dermographism. Compared with controls, differences in HR were not statistically significant.

Atopic dermatitis

The group of AD patients had the highest percentage of individuals showing an enhanced spontaneous HR. From washed leukocytes four patients in the absence of D₂O at 1 h incubation time and nine patients in the presence of 30 % D₂O at 5 h incubation time released more than 15 % histamine. As a group, however, AD patients were not statistically significant from non-allergenic controls.

To investigate the influence of food allergy on spontaneous HR, we divided the AD group into

21 individuals with IgE antibodies to one or more food sources and compared them with 13 patients without any indications of food hypersensitivity, either clinically or by the presence of serum IgE antibodies to food. Statistical analysis did not reveal any difference between the groups. In addition, we were unable to establish any correlation between the total IgE concentration and the degree of spontaneous HR from washed leukocytes. Further, the number of specific sensitizations, as demonstrated by RAST or skin test reactions, did not correlate with the degree of spontaneous HR from washed cells.

In whole blood experiments all patients of the AD group who showed an increase in spontaneous HR both in the absence or presence of D₂O released histamine within the normal range of non-atopic controls.

DISCUSSION

In our experiments we have confirmed spontaneous HR *in vitro* from washed basophils of non-atopic donors to be enhanced in the presence of 30 % D₂O. This enhancement, however, could not be demonstrated in whole blood experiments.

Compared with non-atopic controls spontaneous HR from washed leukocytes in an H₂O and D₂O based buffer was significantly enhanced in patients with allergic rhinitis only. Basically, our results are in accordance with earlier findings of other authors, who, however, besides allergic rhinitis patients, found an enhanced spontaneous mediator release from washed cells in AD patients also (15).

Several other groups have reported enhanced spontaneous HR from washed leukocytes in the presence or absence of D₂O also in asthmatic patients. These findings led to the assumption, that this phenomenon might reflect one of the intrinsic cellular defects leading to or correlating with bronchial hyperreactivity in asthmatic patients (13). Two aspects of our experiments appear to indicate that this may not be the case: firstly there is only a subgroup of asthmatics tending to show an increased spontaneous mediator release from washed cells. Secondly, even for this group, enhanced "releasability" in the absence or presence of D₂O is not observed in whole blood experiments.

Recently (8), it has been reported that spontaneous release of histamine by bronchoalveolar lavage cells in asthmatic patients is not different from healthy controls.

Other authors have tested D₂O concentrations up to 80% and have found an enhanced "histamine release" from washed leukocytes. However, they did not prove that the viability of the cells was still unchanged. Since D₂O at a concentration of 80% has been shown to be cytolytic in a number of individuals, these experimental conditions may not be appropriate in studying phenomena with *in vivo* relevance (3).

There are a number of possible explanations for the differences in spontaneous HR between washed cells and whole blood experiments. Firstly, an increased catabolism of released histamine in whole blood might theoretically be responsible for the differences observed. Secondly, it has been speculated that basophil desensitization may occur in whole blood. Finally, washed basophils might be activated during sedimentation. Kazimierczak et al. (6) have presented evidence for basophil activation tak-

ing place during the washing process. In the same study, it has been shown that spontaneous HR may be inhibited by serum at an optimum concentration of 10%.

From our whole blood experiments we conclude that increased intravascular histamine secretion from basophils *in vitro* is unlikely to be a relevant mechanism for the pathogenesis of asthma or atopic diseases *in vivo*. One may speculate that basophils during the late phase of an allergic inflammation, when they leave the blood vessels and emigrate into the paravascular tissue (1), develop activities which are controlled intravascularly. However, in the absence of any stimulus it appears unlikely that an enhanced spontaneous mediator release of basophils is a relevant mechanism *in vivo*.

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Address:

Prof. Dr. U. Wahn
Univ. Children's Hospital
Freie Universität Berlin
Heubnerweg 6
D-1000 Berlin 19
W. Germany

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