

Evaluation of the human basophil degranulation test using the commercially available Baso-kit as a test of immediate-type hypersensitivity in hay-fever sufferers

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Summary

The basophil degranulation test (BDT) has failed to gain popular acceptance as a test of immediate-type hypersensitivity since its introduction in clinical medicine in 1961. Recent modification of this technique, however, seemed to have increased its reliability. We have studied a group of twenty-five hay-fever sufferers and a similar age- and sex-matched group of healthy controls to compare the sensitivity of a newly available commercial BDT kit (Baso-kit, Laboratoire des Stallergenes) with specific serum IgE levels and skin-prick tests to meadow-grass pollen. A quantitative correlation was found between the positive BDT (> 30% degranulation) and the skin tests, whereas only a qualitative relationship was obtained with specific IgE concentrations. The BDT also identified two subpopulations of responders and non-responders among a third group of hay-fever subjects who had previously been hyposensitized to mixed grass pollen. We conclude that the modified BDT provides a worthwhile addition to the *in vitro* testing of immediate-type hypersensitivity states.

Introduction

The human basophil degranulation test (BDT) originally described by Shelley and Juhlin (1961) offers several distinct advantages as a test of immediate-type hypersensitivity. It provides a direct index of effector-cell response in contrast to the measurement of specific serum IgE concentrations (Platts-Mills, 1979) and to the *in vitro* histamine release by basophils (Lichtenstein & Olser, 1964; Siraganian, 1977; Siraganian & Hook, 1983). It is also relatively inexpensive and simple to perform technically. This test could be particularly useful to study immediate-type hypersensitivity states caused by non-IgE-mediated mechanisms as might occur with anaphylactic IgG antibodies (Parish, 1970, 1978) or in certain drug allergies (Benveniste, 1981; Schlumberger, 1983). To date there is a striking lack of publication, notably of controlled studies in the British literature assessing the usefulness of BDT in clinical practice (Platts-Mills,

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1981, 1982; Kerr & Ganderton, 1983). The availability of a commercial BDT kit based on a modified version (Benveniste, 1981) of the original technique has prompted us to compare this method in defined groups of hay-fever sufferers with the measurement of specific serum IgE levels and skin-prick tests to meadow-grass pollen.

Materials and methods

Subjects

Twenty-five hay-fever sufferers, not previously hyposensitized (Group I) and twenty-five control individuals (Group II), age- and sex-matched, were recruited from a cohort of otherwise healthy medical students. Eighteen hay-fever patients who had previously been hyposensitized (Group III) were also selected for this investigation. All hay-fever subjects had experienced symptoms of rhinitis during the previous months of May to July. The study was carried out between December 1982 and April 1983 to avoid the pollen season in Scotland. Non-steroidal anti-inflammatory drugs, antihistamines, disodium cromoglycate or corticosteroids were avoided for at least 1 week before testing.

Skin testing

All subjects were skin-tested with meadow-grass pollen alone and a mixture of twelve grass pollens including that of meadow grass (Group B2 cat. No. 4100, Bencard Ltd, Brentford, Middlesex). The skin-prick test was performed over the volar aspect of the forearms in a standard manner by two of the authors and read after 15 min. The diameter of the weal was recorded in mm and considered to give a positive test if in excess of 3 mm (Bock *et al.*, 1978).

Specific IgE measurement

Venous blood was collected from each subject at the time of skin testing and coded before dispatch to the laboratory. Serum was immediately stored at -20°C . At the end of the study, specific IgE to meadow-grass pollen (*Poa pratensis*) was measured by batch testing using the RAST method according to the manufacturer's directions (Phadebas IgE-RAST, Pharmacia Diagnostica). Specific IgE concentrations >0.7 units/ml were considered to be abnormally elevated.

Allergen-specific human basophil degranulation test (BDT)

This test was performed according to the method of Benveniste (1981) using the commercially available kits for a mixture of five grass pollens. The mixture contains meadow-grass, rye-grass, cocksfoot, sweet-vernal-grass and timothy-grass pollens (Baso-kit, Laboratoire des Stallergenes, Fresnes, France).

In brief, 20- μl samples of heparinized blood (or buffy-coat preparation) from each subject were placed in parallel rows of microtitre plate wells coated with doubling dilutions of the allergen mixture, except for two control wells which were left uncoated. The plates were then incubated for 10 min at 37°C . 180 μl of toluidine-blue-containing solution were added to each well, and after thorough mixing part of the cell suspension was withdrawn and counted for basophils in a haemoglobinometer. Only

the granulated basophils stained red against a clear background. Percentage degranulation was calculated as follows:

$$\frac{\text{Basophil number in control sample} - \text{basophil number in test sample}}{\text{Basophil number in control sample}} \times 100.$$

BDT was considered positive if more than 30% of at least forty basophils counted had degranulated.

Serum IgG₄ measurement. Total serum IgG₄ concentrations was measured by the modified micro-Mancini technique in all previous hyposensitized hay-fever patients, a group of ten non-hyposensitized hay-fever and ten control subjects chosen randomly, using commercially available monoclonal antiserum to IgG₄ (Seward Labs, Bedford, U.K.).

Results

Table 1 shows the characteristics of the groups at entry. The previously hyposensitized hay-fever patients were slightly older and had higher basophil counts in both their whole-blood and buffy-coat preparations.

With Groups I and II taken together, a highly significant quantitative correlation was obtained between the percentage basophil degranulation and the weal diameters to both meadow-grass pollen alone (Fig. 1) and the mixed-pollen preparation (Fig. 2) ($r = 0.76$ and 0.79 , respectively, $P < 0.001$). Moreover, close concordance was obtained in the skin response to both antigenic preparations (Fig. 3).

No quantitative relationship was found between the percentage basophil degranulation and serum IgE levels, or between the latter and the skin weal diameters. Yet when the results of the BDT, serum IgE and skin-prick tests were considered qualitatively as either positive or negative, all three methods had comparable specificities and sensitivities in the diagnosis of hay fever (Table 2). Four hay-fever subjects from Group I had at least one negative test whereas six control subjects had at least one positive test (Table 3). Interestingly, the two control subjects who had all three tests positive also had a strong family history of atopy. Of note, no correlation was found between the BDT and either the total white cell count or the blood basophil count.

Table 4 shows the results of total serum IgG₄. The range of values varied widely in all three groups with no significant difference between controls, non-hyposensitized hay-fever sufferers and previously hyposensitized hay-fever patients.

It is interesting to note that although patients who had previously been hyposensitized were all strongly skin-test-positive, they fell into two distinct subgroups according to their BDT (Fig. 4). No difference in serum IgE levels was found between the BDT-positive and -negative patients.

Discussion

Despite the relative simplicity and theoretical advantages of the BDT (Klopstock, Schwartz & Grinberg, 1962; Hirsch & Zastrow, 1972) as a measurement of immediate-type hypersensitivity, failure of this method to gain more popular acceptance is probably due to technical difficulties in achieving reproducibility. Recently, however, two research groups in France (Dry, Leynadier & Luce, 1979; Benveniste *et al.*, 1977) have independently reported modifications of the original BDT

Table 1. Characteristics of subjects in study and their haematological findings

	Total number (males: females)	Number of subjects with:			Hb (g/dl)	Total white blood count ($\times 10^9/l$)	Basophil count/mm ³	
		Age (years)	Bronchial symptoms	Family history of atopy			Whole blood	Buffy coat
Non-hyposensitized hay-fever subjects (Group I)	25 (10:15)	21.3 $\pm$ 1.9	10	14	14.1 $\pm$ 3.8	8.7 $\pm$ 5.4	15.9 $\pm$ 5.6	46.6 $\pm$ 13.2
Normal controls (Group II)	25 (10:15)	23.3 $\pm$ 4.1	0	6	14.1 $\pm$ 2.1	8.2 $\pm$ 2.4	18.8 $\pm$ 18.5	48.6 $\pm$ 17.6
Hyposensitized hay-fever patients (Group III)	16 (8:8)	29.2 $\pm$ 9.9	4	8	15.3 $\pm$ 1.3	8.7 $\pm$ 3.2	28.4 $\pm$ 20.2	70.4 $\pm$ 30.8

Mean $\pm$ s.d.

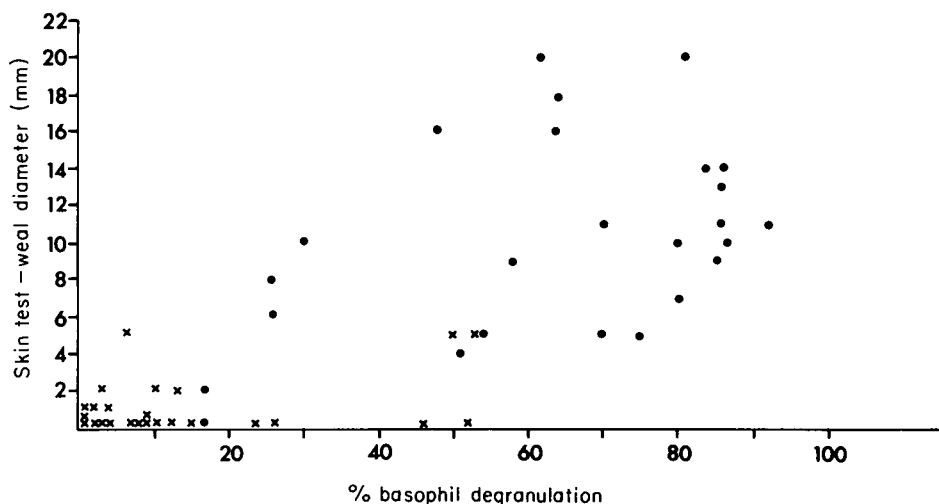


Fig. 1. Percentage basophil degranulation and skin testing to meadow-grass pollen (*Poa pratensis*) in subjects with hay fever (●) and normal controls (×). Linear regression analysis showed a highly significant correlation ($r=0.76$, $P<0.001$).

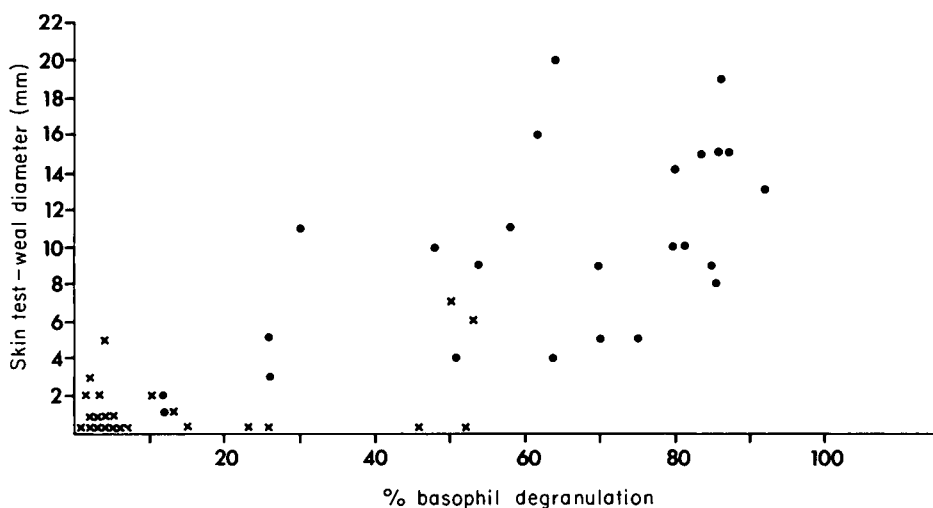


Fig. 2. Percentage basophil degranulation and skin testing to mixed-grass pollens in subjects with hay fever (●) and normal controls (×) ($r=0.79$, $P<0.001$).

which they claimed to be more reliable in clinical practice. In fact, Benveniste's method has already been exploited commercially to diagnose anaphylactic and anaphylactoid reactions to a large variety of allergens and external agents (Benveniste, 1981). Because of the lack of published controlled studies in the evaluation of the BDT which is already finding its way into clinical practice (Mumcuoglu & Wortmann, 1980; Leynadier & Luce, 1980; Leynadier *et al.*, 1980; Pirotzky *et al.*, 1982), we decided to compare this technique using the commercial kits available with other established

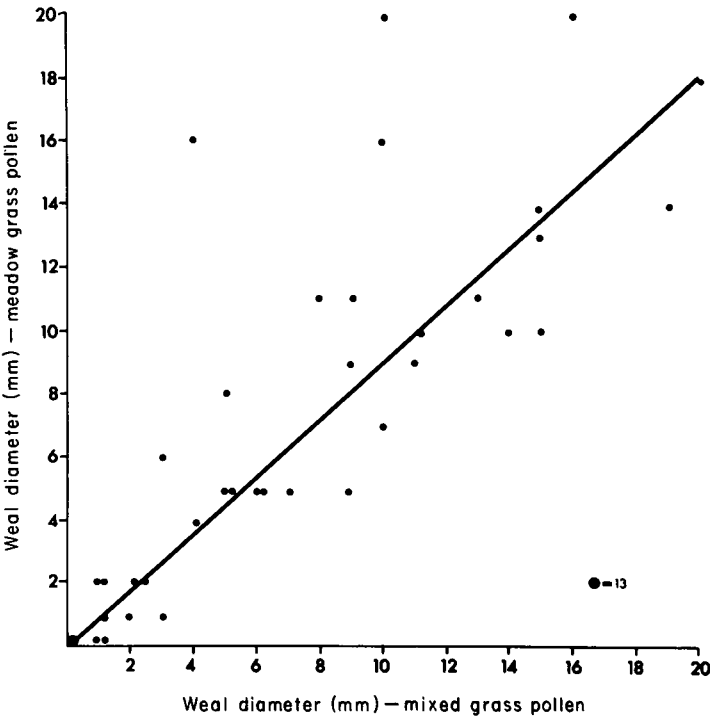


Fig. 3. Weal diameters of skin testing to meadow-grass and mixed-grass pollens in subjects with hay fever and normal controls ($r=0.87$, $P<0.001$).

Table 2. Comparison of results for skin-prick test, BDT and specific IgE concentration to meadow-grass pollen in the diagnosis of hay fever

	Hay-fever subjects (n=25)		Control (n=25)		Specificity (%)	Sensitivity (%)
	+	-	+	-		
Skin-prick test	24	1	3	22	88	96
BDT	21	4	4	21	84	84
Specific IgE conc.	22	3	4	21	84	88

methods of measuring immediate-type hypersensitivity; namely skin-prick test and specific serum IgE concentrations.

The earlier work of Hirsch and Zastrow (1972) and Soifer and Hirsch (1975) had shown concordance between their BDT and skin-test results using inhalant and food allergens. Our data now confirm this quantitative correlation also previously noted between the BDT and the skin-weal diameters in patients with hay fever (Dry *et al.*, 1979). But we only found a qualitative rather than quantitative relationship between specific IgE concentrations to meadow-grass pollen and the BDT, and between the

Table 3. Individual discrepant results for skin tests, basophil degranulation test and specific serum IgE to meadow-grass pollen in Groups I and II

Hay-fever subjects (Group I) <i>n</i> = 5			Controls (Group II) <i>n</i> = 6		
Skin test	BDT	Serum IgE	Skin test	BDT	Serum IgE
+	—	—	+	—	+
—	—	+	—	—	+
+	—	—	—	+	—
+	+	—	—	+	—
+	—	+	+	+	+
			+	+	+

Table 4. Total serum IgG₄ concentrations in subgroups of subjects studied

	Non-hyposensitized hay-fever subjects	Normal controls	Hyposensitized hay-fever patients	
			+ ve BDT	— ve BDT
Number	10	10	7	9
Median	684	493	442	386
(range)	(90–2549)	(90–1309)	(90–832)	(213–832)

No significant difference between any of four subgroups.

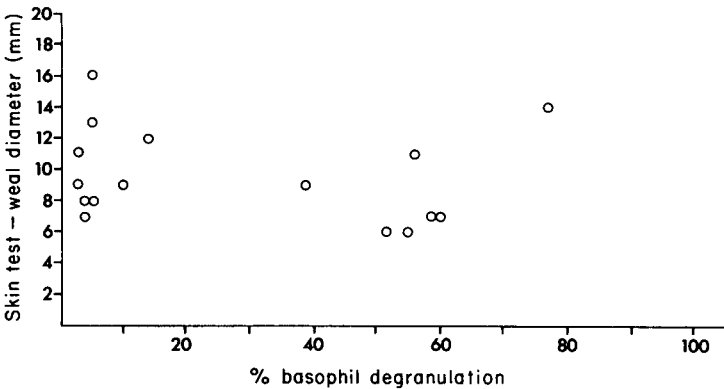


Fig. 4. Percentage basophil degranulation and skin testing to meadow-grass pollen in patients who had previously been hyposensitized ($r = -0.188$, N.S.).

former and the skin tests. This is explainable on the basis of the strong affinity and prolonged attachment of IgE molecules to circulating basophils and tissue mast cells (Kulczycki, Isersky & Metzger, 1974; Kulczycki, 1981) with the result that circulating IgE levels only indirectly reflect the potential reactivity of these effector cells to antigenic stimulation.

Indeed, immediate-type reactions can occur in individuals with no detectable circulating specific IgE (Benveniste *et al.*, 1977). Interestingly the close skin response obtained with meadow-grass pollen alone and the mixture of grass pollens supports the concept of an all-or-none phenomenon operating in mast-cell degranulation following exposure to specific antigen (Pruzansky, Zeiss & Patterson, 1980), since hay-fever subjects from the Glasgow area have a high prevalence of multiple-pollen allergy. The BDT was technically easy to perform according to the manufacturer's directions. It took about 30–45 min for trained technical staff to test the basophils of each individual to one particular antigen; but after five such tests eye fatigue set in. Measurement of basophil degranulation by an automatic image analyser (Leynadier *et al.*, 1981) and by flow cytometric analysis—e.g. using Technicon Hemalog D—is now possible (Nakagawa, Moyseyenko & de Weck, 1981; Benveniste, 1981; Van Toorenenbergen *et al.*, 1982) but unless the technique is in great demand expense is considerable.

In this study the BDT gave four false negatives among the twenty-five hay-fever subjects and four false positives among the controls, thus giving a sensitivity and a specificity of 84% respectively.

The separation of the previously hyposensitized hay-fever patients into distinct positive and negative subgroups despite strongly positive skin tests indicate that the reactivity of peripheral basophils to antigenic degranulation might be different from that of tissue mast cells. The observations might be explained by the preferential binding of specific blocking IgG₄ (Heiner *et al.*, 1980), or IgD antibodies (Bringel *et al.*, 1982) elicited by desensitization therapy to the circulating basophils rather than to the less accessible dermal mast cells. Although no difference in total serum IgG₄ was found between the three groups an increase in specific IgG₄ blocking antibody might still have occurred in the BDT-negative patients. Clearly further investigation is required to determine whether the BDT might have any predictive value in the clinical response to desensitization therapy.

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