

Plasma of normal, but not atopic persons reducing basophil degranulation induced by anti-IgE

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Summary

The response to anti-IgE serum of basophils from allergic and normal persons in the presence of whole plasma or as washed cells in Tyrode solution was examined to detect any inhibiting activity of human plasma. A factor reducing the potential of anti-IgE serum to degranulate basophils was present in plasma of normal but not of allergic persons. It is considered that this property of plasma could contribute to homeostatic control in immediate hypersensitivity.

Introduction

When examining basophil degranulation induced by tetanus toxoid, in subjects with specific IgE against this antigen, we observed that the degranulation index increased if the test was performed in washed blood (Miadonna *et al.*, 1980).

Interference by plasma in the basophil degranulation, in these experimental conditions, seems not to be due to the specific IgG antibodies, with a 'blocking' activity. Specific antitoxoid IgG, added to the washed blood cells, to reconstitute the starting concentration, did not modify the result of the basophil degranulation test with tetanus toxoid. We therefore concluded that an antigen non-specific plasma factor interfered with the *in-vitro* basophil degranulation (Miadonna *et al.*, 1980). A similar observation has been made of the interference activity of plasma for rabbit basophils tested with antigen (Benveniste *et al.*, 1977).

In our investigation we compared the response to an anti-IgE antiserum of the basophil cells of persons with immediate-type allergic disease with the response of basophils from normal subjects. The degranulation test was done both in presence of the autologous plasma and after washing the cells and resuspending them in Tyrode's buffer.

Materials and methods

Patients

We selected ten normal subjects aged between 25 and 35 years, and thirteen allergic subjects (nine with asthma, three with rhinitis and one with acute urticaria) of the same age.

Total IgE dosage

Total IgE levels in the serum were measured by means of PRIST (Pharmacia, Uppsala, Sweden), according to the technique of Ceska & Lundqvist (1972).

Specific IgE dosage

The specific IgE antibodies for the most common allergens (grass pollen, house dust mite, *Alternaria*, *Mucor*, *Aspergillus*, *Cladosporium*, *Penicillium*, dog and cat epithelia, milk, egg white, peanuts, hazel nuts, almonds, wheat and cod) were measured by means of RAST (Pharmacia, Uppsala, Sweden), according to the technique of Wide, Bennich & Johansson (1967).

Basophil degranulation test

The basophil degranulation test was performed according to the technique described by Benveniste *et al.*, (1977). The basophils are the only cells that present a red-brown cytoplasm after metachromatically staining with toluidine blue. After degranulation, the cytoplasm of the basophil faintly stains light blue, as for the other leukocytes. Thus degranulation after an antigen challenge can be evaluated by counting the remaining basophils with red-stained cytoplasm. The degranulation test was performed both on the whole blood in heparin (12.5 u/ml) and on the washed blood. In this instance the blood was collected in heparin and in EDTA (1×10^{-4} mol) and then washed three

Table 1. Results of the basophil degranulation test in whole blood and in washed blood (expressed as % of degranulation) and of the total IgE determination in the normal subjects.

Total IgE (iu/ml)		Dilution of the anti-IgE antiserum									
		1/1		1/10		1/100		1/1000		1/10000	
		A	B	A	B	A	B	A	B	A	B
1) G.A.	12	70	70	60	60	30	57	18	7	0	0
2) G.D.	5	70	84	50	61	28	54	21	7	0	0
3) M.A.	40	77	77	38	61	0	38	0	0	0	0
4) R.G.	140	100	100	77	92	38	75	30	50	0	0
5) L.F.	350	70	86	47	93	30	46	0	46	0	0
6) B.G.	350	100	100	54	75	54	75	50	75	0	0
7) B.A.	135	50	50	28	46	5	40	0	0	0	0
8) M.F.	50	60	90	40	60	40	60	0	0	0	0
9) B.P.	32	70	77	78	80	21	54	7	46	0	0
10) O.A.	70	80	50	75	30	53	10	24	0	0	0
Mean		73	81	52	70	27	55	13	25		
s.d.		15.6	14.7	16	15.2	15.9	12.6	16.5	26.9		
P				<0.05		<0.05					

A: whole blood.

B: washed blood.

Table 2. Results of the basophil degranulation test in whole blood and in washed blood (expressed as % of degranulation) and of the total IgE determination in the allergic subjects.

	Total IgE (iu/ml)	Dilution of the anti IgE antiserum									
		1/1		1/10		1/100		1/1000		1/10000	
		A	B	A	B	A	B	A	B	A	B
1) S.P.	120	78	60	44	60	44	30	0	0	0	0
2) D.F.	75	50	48	44	41	5	0	0	0	0	0
3) M.P.	35	80	73	70	81	30	27	0	0	0	0
4) G.M.	190	78	84	50	46	21	7	28	0	0	0
5) R.A.	25	65	65	50	50	20	20	0	0	0	0
6) S.F.	1000	68	84	40	52	10	13	0	0	0	0
7) S.A.	500	62	60	56	46	31	26	0	0	0	0
8) G.G.	1000	59	66	65	66	30	50	23	42	0	0
9) P.R.	510	69	61	42	44	31	20	0	0	0	0
10) C.M.	120	50	57	50	42	0	0	0	0	0	0
11) V.M.	1000	86	100	64	66	57	66	42	50	0	0
12) C.F.	100	87	61	33	43	26	21	0	0	0	0
13) S.L.	1000	82	79	53	63	0	0	0	0	0	0
Mean		70	69	51	54	23	21				
s.d.		12.6	14.2	10.7	12.3	16.8	19.5				
A: whole blood.											
B: washed blood.											

times with a Tyrode's buffer pH 7.4, according to Findlay, Lichtenstein & Grant (1980), containing 137 mM NaCl, 5.5 mM dextrose, 2.7 mM KCl, 0.36 mM NaH₂PO₄, 12 mM NaHCO₃, using the centrifuge at 500 g at 4°C for 10 min each time. The washed cells were returned to their initial volume in a Tyrode's buffer containing 1.8 mM CaCl₂ and 0.5 mM MgCl₂.

In some instances the cells, washed twice in a Tyrode's buffer without CaCl₂ and MgCl₂, were resuspended in the plasma of other normal or allergic subjects, in a volume equal to the original. The basophil degranulation test was then done. To induce the basophil degranulation increasing dilutions of an anti-IgE antiserum (Behringwerke) were used. The IgG antibodies were purified from this antiserum using the affinity chromatography with protein A Sepharose CL-4B (Pharmacia, Uppsala, Sweden). The final concentration of the purified antiserum was 0.01 of the original. Five µl of serial tenfold dilutions of the anti-IgE antiserum were distributed in the wells of a micro-titre Cooke plate. Twenty µl of whole blood or washed blood cells were then added in each well and gently mixed with the antigen. After incubation for 10 min at 37°C, 180 µl of 0.025 toluidine blue in 30% ethanol, brought to pH 3.5 with glacial acetic acid, were added and gently mixed with blood. The basophil cells were counted in a Fuchs-Rosenthal haemocytometer chamber.

Subjects with less than 50 basophils/mm³ were not examined.

The test was read 'blind'. Tests were carried out in duplicate and the mean value of both counts taken. The percentage of degranulation was calculated using the following formula:

$$\frac{\text{Bas. No. before Ag challenge} - \text{bas. No. after Ag challenge}}{\text{bas. No. in control samples}} \times 100; (\text{bas.} = \text{basophil}).$$

Statistical analysis

The data of the basophil degranulation test in whole blood and in washed blood cells were compared by means of Wilcoxon's test.

Results

In normal persons the RAST for the antigens tested was always negative and the total IgE levels ranged between 5 and 340 iu/ml (mean 122 ± 124.3). It is realized that the mean value is well above the generally accepted 15–20 iu/ml for normal persons. However, our results are in accord with the mean IgE levels observed in the healthy Italian population (Bonini *et al.*, 1982). In all the allergic subjects the RAST was positive for one or more allergens, and the total IgE levels ranged between 25 and 1000 iu/ml (mean 440 ± 417). The percentage of basophil degranulation with the same amount of anti-IgE antiserum varied from subject to subject and was not related to total IgE levels or to basophil number, as reported by Sobotka *et al.*, (1978). The normal subjects showed a significant difference between the degranulation in

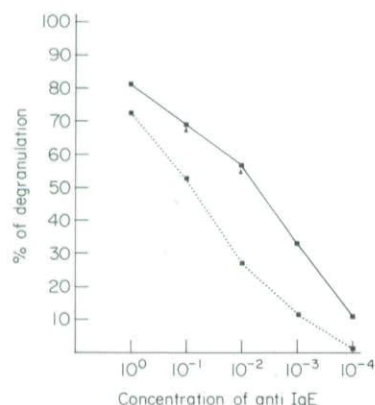


Fig. 1. Normal subjects. Comparison between the means of the basophil degranulation indices in whole blood (● · · · · ●) and in washed cells (● — ●). The increase of the indices in washed cells is evident ($\Delta P < 0.05$).

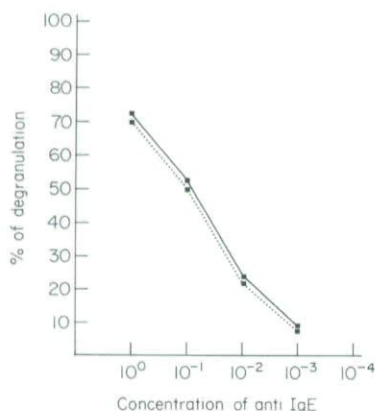


Fig. 2. Allergic subjects. Comparison between the means of the basophil degranulation indices in whole blood (● · · · · ●) and in washed cells (● — ●).

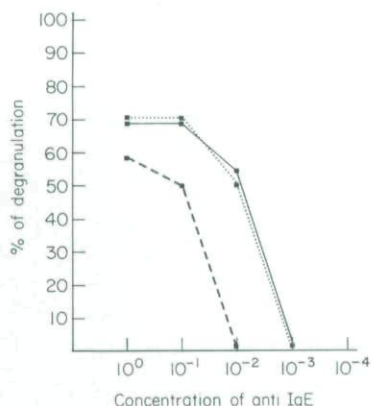


Fig. 3. Normal subject's cells in autologous plasma (●---●); in Tyrode's buffer (●····●); and in allergic plasma (●—●). Mean of the basophil degranulation indices in two normal subjects compared with the mean of those obtained by suspending the same subjects' cells in the plasma of an allergic subject.

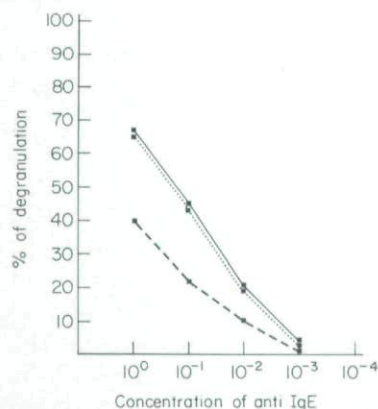


Fig. 4. Allergic subject's cells in autologous plasma (●····●); in Tyrode's buffer (●—●), and in normal plasma (●---●). Mean of the basophil degranulation indices of two allergic patients in whole and in washed blood, compared with the mean of those obtained by suspending the cells of the same subjects in the plasma of a normal subject.

autologous plasma and in Tyrode's buffer, where a significant increase of the degranulation indices was observed ($P < 0.05$) (Fig. 1). In contrast, in the allergic subjects, the degranulation curves in autologous plasma and in Tyrode's buffer overlapped perfectly (Fig. 2).

In order to confirm this result we did the following experiments: (1) the cells of two normal subjects were resuspended in the plasma of an allergic subject (Fig. 3). A good correlation was observed between the degranulation curve obtained in these conditions and that of the cells suspended in Tyrode's buffer. Both were higher than the degranulation curve of the cells in autologous plasma. (2) the cells of two allergic subjects were respectively suspended in the plasma of two normal subjects. In these conditions we observed a decrease in the numbers of the degranulating cells (Fig. 4), when compared to the degranulation curve in autologous plasma and in Tyrode's buffer, which were coincident with one another.

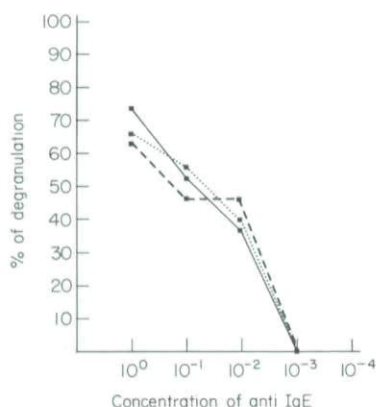


Fig. 5. Allergic subject's cells in autologous plasma (● · · · · ●), in Tyrode's buffer (● ——— ●), and in allergic plasma (● — — — ●). Comparison between the means of the degranulation indices of two allergic patients compared with the mean of those obtained by suspending the cells of the same subjects in the plasma of an allergic patient.

(3) the cells of two allergic subjects were resuspended in the plasma of two other allergic subjects. In this experiment we observed a degranulation curve that overlapped perfectly the one obtained in autologous plasma (Fig. 5).

Discussion

These data indicate that in the normal person there is a plasma factor that can interfere with basophil degranulation induced by anti-IgE. This factor appears not to be antigen-specific, and not due to blocking IgG antibodies. In the allergic subjects we did not observe any difference between the degranulation in the presence of autologous plasma or Tyrode's buffer; indeed the two curves of activity overlap throughout. The blocking activity of plasma, observed in the normal subjects either after stimulating the basophils with an anti-IgE antiserum as in this case, or after an antigen challenge in the man (Miadonna *et al.*, 1981) and in the experimental animal (Benveniste *et al.*, 1977), seems to be absent from plasma of allergic persons.

The degranulation curve observed in cells of a normal person suspended in the plasma of an allergic patient overlaps perfectly the curve of the cell response when they are suspended in Tyrode's buffer. Furthermore, if the cells of an allergic subject are suspended in the plasma of a normal person a decrease is obtained in the degranulation, in comparison with the one obtained in autologous plasma, which overlaps that of a Tyrode's buffer diluent. If the cells of an allergic subject are suspended in the plasma of another allergic subject, no modification of the degranulation test is observed.

The intensity of the degranulation varies from subject to subject. Moreover many factors, i.e. the age of the patient (Marone & Condorelli 1981) or a 'chronic' degranulation, as it happens in chronic urticaria (Kern & Lichtenstein 1976), can affect the releasability of the mediators from the basophils. These features do not appear to influence the phenomena we describe.

Siraganian & Levine (1975) described in a patient allergic to seminal plasma a serum activity that enhanced the histamine release from the basophils. This author did not confirm this finding in other cases. Our preliminary data encourage us to further

study the 'blocking' activity of plasma which could play a homeostatic role in the immediate hypersensitivity.

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