

Release of Histamine from Human Leukocytes by One Preparation of IgG Oligomers*

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In preliminary experiments aimed at investigating the effect of covalently cross-linked human myeloma subclass proteins on histamine release from human leukocytes, we observed one preparation (designated here IgG-HR) made from pooled, purified immunoglobulin G which consistently released histamine from these cells. Dimers and trimers, but not monomers isolated from columns of Sephadex G-200 and Ultrogel AcA22 following incubation of immunoglobulin G (Nordic Laboratories) with dimethyl suberimide, released histamine from cells of all donors tested. In contrast, cells from the same donors showed variable responsiveness to dimers of IgE (prepared by similar techniques) or to anti-IgE. IgG-HR failed to release histamine from a "basophil-rich" mononuclear cell preparation depleted of most of the erythrocytes, platelets, neutrophils and eosinophils by centrifugation through a Ficoll-Hypaque cushion. The data suggest that IgG-HR was releasing histamine indirectly from basophils by first interacting with another cell. IgG oligomers prepared from different sources of pooled, purified IgG failed to release histamine. Although we did not have sufficient IgG-HR to adequately define this releasing activity, we feel that the data represent a potentially novel, if rare, mechanism of mediator release involving basophils and another cell.

Key words: cross-linking reagents; histamine release; IgG; leukocytes.

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In both human and animal experimental models, several non-IgE-dependent mechanisms of mediator release have been described. These mechanisms include release by anaphylatoxins (9, 26), drugs such as aspirin (29) and codeine (3, 28), radiocontrast compounds (22), and anesthetic agents (32). The administration of immunoglobulin concentrates also occasionally gives rise to anaphylactoid reactions (2). Reactions such as these emphasize the potential importance of alternative, non-IgE-

dependent pathways of triggering basophils or mast cells.

During experiments designed to test the effects of human IgG myeloma subclass oligomers on histamine release from human leukocytes, we found a preparation of purified immunoglobulin which, after cross-linking, consistently released histamine from washed leukocytes. Cell fractionation studies suggested that the oligomers did not release histamine directly from the basophil, but required the presence of

Abbreviations: DMS: dimethyl suberimide; EDTA: ethylenediamine tetraacetic acid; D₂O: deuterium oxide; PAGE: polyacrylamide gel electrophoresis; IEP: immunoelectrophoresis; PRIST: paper radioimmunosorbent test; HEPES: N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid.

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another cell. Although other oligomer preparations prepared in the same manner failed to release histamine, we feel that our data represent a potentially very interesting, if rare mechanism of release from basophils which might have important implications in certain types of allergic reactions.

MATERIAL AND METHODS

Histamine release from human leukocytes

Venous blood from healthy volunteers was mixed with dextran and EDTA and left at room temperature for 90 min (15). The resulting plasma supernatant solution was removed, centrifuged at $110 \times g$, the leukocytes washed twice with HEPES buffer, and then finally suspended in HEPES buffer containing 0.6 mM CaCl_2 and 1 mM MgCl_2 . A final concentration of 40% D_2O was used in all experiments. One-half ml of cells was added to tubes containing 0.5 ml of the IgG or IgE oligomer or anti-IgE. Following a 45–60 min incubation at 37°C , the reaction was stopped by centrifugation in the cold and the amount of histamine in the supernatant determined by an automated method (25).

In some experiments, a portion of the blood was collected in heparin, diluted with saline, and layered on to Ficoll cushions according to the method of Böyum (4). The resulting mononuclear fraction contained about 90% lymphocytes, 5–8% mononuclear cells, 1–3% basophils, and less than 2% contaminating neutrophils.

Preparation of immunoglobulin oligomers

This method has been described in detail (13, 24, 33). Briefly, 50 mg of human immunoglobulin G or E was incubated with 30-fold molar excess of dimethyl suberimidate at 30°C for 3 or 2 h, respectively. The oligomers were separated from the monomeric material and columns of Sephadex G-200 and Ultrogel AcA22 connected in tandem, and the elution profile determined by absorption at 280 nm. The monomeric material was rerun on the same columns to minimize contamination with the heavier material.

Reagents and buffers

The following reagents were purchased: Dimethyl suberimidate (DMS), Sigma Chemical Company, St. Louis, Missouri, USA; EDTA and D_2O , Fluka AG, Switzerland; DEXTRAN T 70 Pharmacia Diagnostics AB, Uppsala, Sweden; sheep anti-human IgE (Fc) and purified human IgG (No. 29-681), Nordic Immunological Laboratories, Tilburg, The Netherlands. PRIST Kits (Phadebas IgE PRIST[®]), were provided by Pharmacia, Zurich and IgG Sandoglobulin[®]/Immunoglobulin SRK by the Swiss Red Cross, Berne, Switzerland. Another purified IgG sample was provided by Dr. F. Skvaril of Berne and a sample of IgA was supplied by Nordic Laboratories. Dr. T. Ishizaka provided the human IgE (PS). The following buffers were used for histamine release and the cross-linking reaction respectively: $1 \times$ HEPES: 20 mM HEPES containing 5 mM KCl, 125 mM NaCl and 5 mM glucose, pH 7.4; Tris-HCl: 0.2 M Tris, pH 8.6. The columns were eluted with $1 \times$ HEPES without added glucose.

RESULTS

Dimers and trimers of IgG-HR at concentrations above $1 \mu\text{g}/\text{ml}$ in the presence of 40% D_2O released histamine from washed leukocytes (Fig. 1, right). In other experiments, 3–5 μg of IgG-HR/ml elicited release. Ten-fold lower concentrations of IgE dimer and trimer (also in the presence of 40% D_2O) released histamine in the same experiment (Fig. 1, left) whereas monomeric IgG or IgE did not.

To see if the histamine releasing activity of these IgG oligomers was a common feature of all cross-linked IgG, we used other purified immunoglobulin preparations as starting material for the reaction with DMS. Dimers and trimers made from a second vial of Nordic IgG having the same lot number as the first, or from two purified IgG samples, did not release histamine from washed human leukocytes (Fig. 2). Only the original Nordic dimers and trimers, designated here IgG-HR, had histamine releasing activity.

We were concerned that this activity might

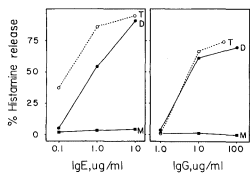


Fig. 1. Histamine release from washed human leukocytes by oligomers of IgE (left) and IgG-HR (right). T = trimer; D = dimer; and M = monomer.

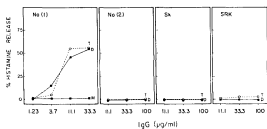


Fig. 2. Ability of dimers (D) and trimers (T) of different immunoglobulin preparations to release histamine from washed leukocytes. From left to right, immunoglobulins obtained from Nordic Laboratories No. (1) and No. (2), from Dr. Skvaril in Berne (SK) and from the Swiss Red Cross (SRK).

be due to contamination with IgE, but PRIST analysis revealed that the original material contained only 16 ng of IgE/mg IgG or about 500 times less IgE than required for histamine release by human IgE dimer (13 and Fig. 1).

Comparing maximum percent histamine release by IgE dimer, anti-IgE or the IgG-HR dimer suggested different patterns of activity (Table 1). The release, listed in descending order of the response to 10 μ g/ml of the IgE dimer, ranged from 93% to 1%. The anti-IgE response was roughly related to this response: in general the best "releasers" with the IgE dimer were also the best "releasers" upon anti-IgE challenge. In contrast, all of these 14 donors' cells responded to the IgG-HR dimer, with release ranging from 34% to 85%. Release by IgG-HR appeared to be unrelated to

the IgE-dependent responses: those three donors (L.H., Z.P. and P.L.) whose cells released most poorly when challenged with IgE dimer or anti-IgE still released histamine with the IgG-HR dimer.

During these experiments we noted that in all the samples in which released histamine was measured following challenge with IgG-HR, marked hemolysis of the red blood cells could also be observed. The hemolysis was unique to IgG-HR dimer and trimer, and was not seen with the corresponding monomer, nor with any other preparations of IgE or IgG oligomer. These data suggested that release by IgG-HR was different from the classical IgE-dependent mechanism and that other cells were also affected/involved.

To explore this possibility, we separated cells into a "basophil-rich" mononuclear cell fraction, thus eliminating most of the neutrophils, eosinophils, erythrocytes and platelets. The histamine release from this "basophil-rich" fraction is compared with release from washed leukocytes challenged with IgG-HR dimer, IgE dimer or anti-IgE (Fig. 3). As expected, the dose-response curves from the unfractionated and fraction-

Table 1
Histamine release by dimers of IgG, IgE and by anti-IgE

Donor	Percent histamine release by		
	IgE dimer	Anti-IgE	IgG HR
S.I.	93	87	71
Ro.J.	82	72	63
H.E.	78	80	39
Z.T.	63	82	75
F.J.	61	70	49
Ru.J.	59	87	85
G.M.	43	55	60
N.M.	32	72	71
J.G.	32	27	58
T.E.	31	86	62
R.C.	19	100	72
L.H.	7	14	34
Z.P.	2	18	45
P.L.	1	27	70

Washed leukocytes were incubated in the presence of 40% D₂O with IgE dimers (10 μ g/ml), with anti-IgE (diluted 1/10,000) or with 100 μ g/ml of the IgG HR at 37°C for 45-60 min.

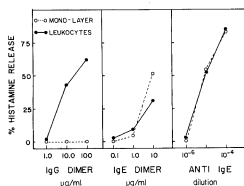


Fig. 3. Histamine release from washed leukocytes (solid lines) or from a basophil-rich mononuclear cell fraction (broken line) challenged with IgG-HR dimer, IgE dimer, or anti-IgE.

ated cells were the same when challenged with IgE dimer or anti-IgE. The IgG-HR dimer, however, did not release histamine from the "basophil-rich" mononuclear fraction, but only from the washed leukocytes. Identical results were obtained in another experiment. Release by the IgG-HR also did not occur in the absence of calcium (data not shown).

IEP analysis revealed no C3 or IgM but trace amounts of IgA in the purified immunoglobulin from Nordic Laboratories. We therefore re-

acted other partially purified immunoglobulins with DMS (Table 2). The whole reaction mixtures were tested directly for histamine release since we had previously determined that 33.3 µg/ml of the purified IgG-HR dimer could release histamine in the presence of 333 µg/ml of the monomeric IgG (data not shown). Modest histamine releasing activity was observed at the highest concentrations tested in some IgG preparations made from pooled sera. Two-fold dilutions of these samples, however, did not release histamine. When sample #8 was passed over the tandem columns 30 µg of dimeric material failed to release histamine from washed leukocytes. Cross-linked IgA also failed to release histamine. We conclude that the histamine releasing activity of IgG-HR oligomers was unique to the original IgG preparation since this activity was not readily demonstrable with other covalently cross-linked immunoglobulin samples prepared under a variety of experimental conditions.

DISCUSSION

The existence of anaphylactic IgG antibody in humans has been clearly established (5, 18, 27)

Table 2
Histamine release by oligomers of partially purified immunoglobulin preparations

Sample ¹ (mg/ml)	Method of preparation	Also contains ²				Percent HR ³			
		IgA	IgM	IgE	C3	1	2	3	4
1 (1.25)	DEAE "batch"	-	-	-	-	0	0		
2 (0.83)	DEAE "batch"	+	-	-	-	0	0		
3 (1.1)	DEAE "batch"	+	-	-	-	0	0		
4 (0.6)	DEAE "batch"	+	-	-	-	0	0		
5 (1.0)	DEAE "batch"	+	-	-	-	0	0		
6 (4.0)	40% ammonium sulfate	+	+	-	+	34	28	14	
7 (2.4)	Ethanol precipitation	+	+	-	-	14	15		
8 (1.6)	Ethanol precipitation	+	+	-	-	2	3	28	5
IgA (0.05)	from Nordic Labs	+	-	-	-	3	2		

¹ Samples 1-5 were sera from individuals with low serum IgE levels; samples 6-8 were pooled sera. Partially purified IgG was reacted with DMS (see Material and Methods), the formation of oligomers confirmed by PAGE and the samples tested for histamine release.

² As analyzed by immunoelectrophoresis: + = present; - = absent.

³ Maximum histamine release by mixtures of immunoglobulin after reaction with DMS. The concentrations tested are given in parentheses after the sample codes.

as has the presence of IgG receptors on human basophils (12). Little is known, however, about the precise mechanism of IgG-mediated human basophil activation, and whether stimulation of IgG receptors leads to histamine release from human leukocytes is not yet entirely resolved (1, 8, 10, 11, 12, 16, 17, 19, 30, 31).

We present here experiments describing histamine release from human leukocytes by dimers and trimers of a covalently cross-linked immunoglobulin G preparation from Nordic laboratories. The activity of this preparation, IgG-HR, had several features not seen in other preparations of IgG oligomers (Fig. 2). IgG myeloma subclass oligomers (33) or IgE oligomers (13, and Fig. 3): 1) The IgG-HR dimers and trimers released histamine from cells of all donors tested; 2) hemolysis of contaminating red cells accompanied histamine release by IgG-HR dimer; 3) histamine release by IgG-HR dimer did not occur in a "basophil-rich" mononuclear cell fraction.

Although contamination of IgG preparations by IgE has been described (23), and we did find IgE in our starting IgG, we are confident that the activity of IgG-HR was independent of IgE-mediated reactions. First, the concentration of IgE (16 ng IgE/mg IgG) was far too low to effect histamine release, assuming that the relative biological activity of cross-linked IgE from the immunoglobulin preparations was similar to that of the cross-linked IgE myeloma protein (13). Second, the cell fractionation studies clearly showed that histamine release was dependent on cells (e.g. neutrophils, eosinophils, erythrocytes, platelets) which were removed by centrifugation through Ficoll. Unfortunately, we did not have sufficient IgG-HR to examine the primary target cell in detail nor were we able to reliably generate IgG-HR in several attempts (Fig. 2 and Table 2).

We cannot eliminate the possibility that the activity of IgG-HR was an artifact either of the reaction with DMS or during the isolation procedure. We have, however, isolated at least seven other preparations of IgG monomers, dimers, trimers, and heavier oligomers (which never released histamine) and have isolated two preparations of IgE oligomers (which do not

release histamine) using three different tandem columns over a period of 8–10 months. There is evidence in other model systems, however, that neutrophil products may activate human basophils (14) and basic granule proteins from rabbit, rat or human neutrophils release histamine from rat mast cells (20). In the rabbit, IgG antibodies increase vascular permeability in skin and release histamine by a mechanism which is dependent on complement and requires platelets and neutrophils (21). It is unlikely, however, that complement was involved in the release induced by IgG-HR. Our experiments were performed with dimers and trimers of IgG in a model using washed leukocytes (i.e. in the absence of serum). We did not test IgG-HR for the presence of endotoxin, recently shown to stimulate neutrophils which are attached to petri dishes (7). Neutrophil stimulation by endotoxin was not observed with cells kept in suspension (6), conditions which more closely approximate the histamine release experiments.

The extent to which these non-IgE-dependent mechanisms may participate in anaphylactoid or idiosyncratic reactions is not entirely clear nor do our data address this question. We present the data, nevertheless, because we feel that it represents an interesting, if rare, mechanism of triggering basophils which involves oligomeric IgG antibody and at least another cell.

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