Complement-Dependent Histaminase Release from Human Granulocytes

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ABSTRACT The role of particle-bound complement proteins in the induction of noncytotoxic enzyme release from human granulocytes was investigated with the use of sera genetically deficient in complement and highly purified complement components. Release of histaminase, one of two important histamine catabolizing enzymes, and β-glucuronidase from polymorphonuclear leukocytes was solely dependent on particle-bound C3b (the larger cleavage product of the third component of complement) when fluid-phase complement was excluded. The extent of enzyme release was a function of particle-bound C3b input, was reduced by exposing the particles to C3b inactivator, and was blocked by fluid-phase C3b. Phagocytosis of the C3b-coated particles was not required for enzyme release from neutrophils.

In contrast, phagocytosis of "opsonized" particles was required for noncytotoxic release of histaminase and arylsulfatase from eosinophils; other proteins, as well as C3b, were able to opsonize particles for induction of enzyme release from eosinophils.

These studies suggest a dual role for complement (particularly C3) in modulating vascular permeability phenomena, i.e., release of vasoactive mediators by the action of C3a and C5a, and release of the corresponding enzymes that inactivate the mediators by C3b.

INTRODUCTION

The serum complement system was discovered in the late nineteenth century because of its cytolytic effect; i.e., ability to induce membrane damage (1, 2). Since then, other important biologic activities of complement have been recognized. These include immune adherence (3), viral neutralization (4), chemotactic (5) and opsonic (6) functions, as well as histamine release from basophils and mast cells (7).

Complement-dependent histamine release is mediated by anaphylatoxins, fragments of the third (C3) and fifth (C5) components of complement. These active polypeptides, C3a (9,000 daltons) (8) and C5a (11,000 daltons) (9), are cleaved from the alpha chains of C3 and C5, respectively. C5a, in addition, has been shown to mediate release of lysosomal enzymes from polymorphonuclear leukocytes, especially in the presence of cytochalasin B (10). The larger fragments of C3 and C5 cleavage, C3b and C5b, participate in further activation of the complement sequence, but, in the case of C3b, also interact with specific cell surface binding sites to effect cellular events (11).

We have previously shown that histaminase, one of two important histamine-catabolizing enzymes in humans, was released from eosinophils and neutrophils during incubation with zymosan opsonized with normal human serum; zymosan prepared with heated serum did not induce histaminase release (12, 13). These experiments were performed under conditions that excluded the action of fluid-phase complement components (e.g., C3a, C5a). This observation suggested the possibility that surface-bound components of the serum complement system might be essential for the preparation of zymosan to effect histaminase release from human leukocytes.

The availability of sera from patients with genetic deficiencies of individual complement proteins and the purified components has made it possible to define the role of complement in opsonized zymosan-induced histaminase release from granulocytes. These studies indicated that noncytolytic enzyme release from polymorphonuclear leukocytes is a result of action of particle-bound C3b at the cell surface and does not require phagocytosis of C3b-coated particles to induce release.

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In contrast, histaminase release from eosinophils is absolutely dependent on phagocytosis; proteins other than C3b are also capable of preparing zymosan for induction of eosinophil enzyme release.

METHODS

Materials. The following were purchased: *Saccharomyces cerevisiae* yeast (zymosan A) (Sigma Chemical Co., St. Louis, Mo.), calcium ionophore A-23187 (Eli Lilly & Co., Indianapolis, Ind.), cytochalasin B (CB[1]; Aldrich Chemical Co., Inc., Milwaukee, Wis.). CB and calcium ionophore were dissolved in dimethyl sulfoxide (Aldrich Chemical Co., Inc.,) and diluted for use (final maximum concentration of dimethyl sulfoxide of 0.5%). This concentration of dimethyl sulfoxide, when added to control cells, did not alter cell morphology nor affect enzyme release or measurement. Phenolphthalein glucuronic acid, phenolphthalein, *p*-nitrocatechol sulfate, imidazole acetic acid, 4-nitrocatechol, phosphotungstic acid, Trizma Base, Trizma HCl (Sigma Chemical Co.), hydroquinone, and cellulose thin-layer chromatogram sheets (Eastman Kodak Co., Rochester, N. Y.), Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.), gelatin (Difco Laboratories, Detroit, Mich.), aminoguanidine (Eastman Chemical Div., Eastman Kodak Co.), Hypaque (Winthrop Laboratories, New York) Ficoll, Sephadex G-75, and Sepharose 4B (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) were obtained in reagent grade. Tris A buffer consisted of 0.025 M Tris, pH 7.35 at 37°C, 0.12 M sodium chloride, 0.005 M potassium chloride, and 0.3 mg/ml human salt-poor albumin (Hyland Laboratories, Costa Mesa, Calif.). The albumin was centrifuged at 100,000 *g* for 30 min before use. Tris ACM contained, in addition, calcium at 0.6 mM and magnesium at 1.0 mM (14). Ring [2-^{14}C]-histamine (58 mCi/mM) (Amersham Corp., Arlington Heights, Ill.) was used after repurification by thin-layer chromatography (15).

Preparation of neutrophils. Whole blood anticoagulated with sodium EDTA (final concentration 5 mM) was diluted with an equal volume of Tris buffer (Tris A without albumin) and centrifuged at 400 *g* for 30 min at 10°C on a 15-ml cushion of Ficoll-Hypaque (16). Purified granulocyte suspensions (<2% mononuclear cells) were separated from erythrocytes in the pellet by 1–2 cycles of hypotonic lysis (17).

Preparation of eosinophils. In some experiments, eosinophils were purified by a modification of the method described by Parrillo and Fauci (18). Granulocyte suspensions were obtained, as described above, from patients with 15–60% eosinophils. The granulocytes were then washed and resuspended in Medium 199 (Microbiological Associates, Walkersville, Md.) with 10% human salt-poor albumin. Neutrophils were removed by incubation of the resultant neutrophil-eosinophil suspension on a nylon wool (Leukopak, Fenwal Laboratories, Inc., Deerfield, Ill.) column in the following manner: 3 g of nylon wool were packed into a 35-ml syringe with a three-way stopcock, and the column was primed with 100 ml of Medium 199 supplemented with 10% albumin. The granulocyte suspension ($1.0–2.0 \times 10^7$ cells in about 5 ml Medium 199 supplemented with 10% albumin), was added to the column and allowed to permeate the entire surface area of the nylon wool by immediately eluting twice with reapplication of the eluant. The column was incubated at 37°C for 15 min to permit binding of neutrophils; the eosinophils were eluted with 50 ml of warm (37°C) Medium 199 supplemented with 10% albumin. The eosinophils were then washed with Tris A and resuspended in Tris ACM for use in each experiment. This resulted in eosinophil suspensions that were >95% viable as determined by trypan blue (Grand Island Biological Co., Grand Island, N. Y.) exclusion, yields of 50% and purity of 80–95%.

Alternatively, eosinophils were purified by the method described by Vadas et al.[2] A granulocyte suspension that contained eosinophils and neutrophils was obtained as described above, from patients with 20–40% eosinophils. The granulocytes were washed, then resuspended in 2 ml Hanks' balanced salt solution without calcium and magnesium, but supplemented with 0.2% gelatin (Microbiological Associates) at a concentration of $\cong 2.5–3.5 \times 10^7$/ml. The cells were layered on 6 ml discontinuous 18–30% metrizamide (Accurate Chemical & Scientific Corp., Hicksville, N. Y.) gradients, which were prepared with Hanks' balanced salt solution without calcium and magnesium, but with 0.2% gelatin and 0.1 mg/100 ml deoxyribonuclease I (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.). After centrifugation at 400 *g* for 40 min at 25°C in 15-ml Falcon tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.), the lower, eosinophil-rich layer, was removed, washed with Tris A, and resuspended in Tris ACM. This resulted in preparations of >90% eosinophils, yields of 50%, and >95% viability by trypan blue exclusion.

Cell incubation and enzyme release procedures. Leukocyte suspensions that contained $0.5–1.0 \times 10^7$ cells in 0.1 ml Tris ACM were mixed with varying concentrations of zymosan, ionophore, or erythrocytes in a final volume of 0.15 ml and incubated 30 min at 37°C with intermittent shaking (17). The reactions were stopped by rapid cooling to 0°C, followed by pelleting the cells at 600 *g* for 10 min at 4°C. The supernatant solutions were decanted, frozen at −90°C, and used later for enzyme determinations. Controls that contained cells and buffer were included in each experiment.

CB was used at 5 μg/ml, a concentration determined in previous experiments (12, 13), to block phagocytosis of opsonized zymosan. In some experiments, parallel studies with high particle-to-cell ratios were performed to confirm the inhibition of phagocytosis by CB.

Viability of cells. Cell viability at the end of each experiment was assessed by either trypan blue exclusion or lactic dehydrogenase release (19). Only experiments in which >90% of the cells remained viable by these criteria are reported.

Preparation of zymosan. Zymosan was opsonized by incubating 10 mg of boiled washed zymosan with 1 ml of fresh normal human serum for 30 min at 37°C, then washing (three times) in Tris A buffer to remove nonadherent serum components. Zymosan was also prepared in a similar manner with sera from patients homozygous for deficiencies of the second, third, fifth, and sixth components of complement (20). The C5- and C6-deficient sera were generous gifts of Dr. J. P. Leddy (Rochester, N. Y.). A reagent deficient in C3 and factor B was prepared by incubating C3-deficient serum for 18 h at 4°C with a cyanogen bromide-activated Sepharose 6B-goat immunoglobulin (Ig)G antihuman factor B affinity resin. The

[1] *Abbreviations used in this paper*: CB, cytochalasin B; CoF, cobra-venom factor; EC3b, erythrocytes from patients with C3b inactivator deficiency; SDS, sodium dodecyl sulfate; Tris A, 0.025 M Tris, pH 7.35 at 37°C, 12 M sodium chloride, and 0.3 mg/ml human salt-poor albumin; Tris ACM, Tris A with calcium at 0.6 mM and magnesium at 1.0 mM.

[2] Vadas, M. A., J. R. David, A. E. Butterworth, N. T. Pisani, and T. A. Siongok. Comparison of the ability of eosinophils and neutrophils to damage schistosomula of *S. mansoni*, as assessed by radioisotopic and microscopic methods. Submitted for publication.

volume of serum after absorption was adjusted to correspond to that of the starting serum according to albumin and IgG content. No factor B was detected immunochemically in the depleted serum indicating that >97.5% of the factor B was removed by this procedure.

Preparation of complement components. Human C3 was purified by the method of Nilsson and Muller-Eberhard (21). C3b-coated erythrocytes obtained from a patient with C3b inactivator deficiency (22) were washed in Tris A, then suspended in Tris ACM, and used without further manipulation. C3b was purified from this patient's plasma by a method for C3 separation (21). The C3b eluted in the later fractions of hydroxylapatite (Bio-Rad Laboratories, Richmond, Calif.). Electrophoretic mobility and immunochemical analysis of this purified material was consistent with its identification as C3b. Cobra-venom factor (CoF) was purified from *Naja naja* venom as described (23). Erythrocyte complement intermediates, EAC1423, were prepared by the method of Rapp and Borsos (24). Highly purified human C3b inactivator was prepared by Dr. F. S. Rosen, as described (25). Human factor B was purified by the method of Boenisch and Alper (26). C3, CoF, and factor B were radiolabeled with ^{125}I by the iodine monochloride technique (27).

Preparation of Sephadex C3b. Sephadex C3b was prepared according to the technique of Arnaout et al. (28). Briefly, normal human serum was dialyzed overnight at 4°C against veronal-buffered saline (pH 7.35) with 10 mM EGTA and 5 mM Mg. Equal volumes of Sephadex G-75 and dialyzed normal human serum diluted 1:4 in veronal-buffered saline-Mg EGTA buffer were incubated at 37°C with gentle shaking. At timed intervals, portions were removed, washed with 150-fold vol of a 2 M NaCl solution, the beads collected by vacuum on Whatman No. 1 filters (Whatman, Inc., Clifton, N. J.), then washed with 50-fold vol of phosphate-buffered saline, pH 7.2. The concentration of particles was adjusted to 1×10^7 beads/ml and used to induce enzyme release.

A small portion of each of the batches of washed Sephadex was resuspended in 4 vol of phosphate-buffered saline with 100 μl dextranase (1 mg/ml) (150 U/mg, Worthington Biochemical Corp., Freehold, N. J.) and shaken for 12 h at room temperature. The mixtures were then centrifuged at 1,500 rpm for 10 min, the supernatant fluid was concentrated 10-fold in collodion bags (Schleicher & Schuell, Inc., Keene, N. H.), then dialyzed against phosphate-buffered saline. The concentrates were analyzed on 1% sodium dodecyl sulfate (SDS)-7.5% polyacrylamide gels according to the method of Laemmli (29).

Enzyme assays. Histaminase was determined by a radiochromatographic assay (15) on cellulose chromatogram plastic sheets (Eastman Kodak Co.) with butanol:concentrated acetic acid:water (4:1:1) as solvent. The area that contained histamine-degradation products was cut and its radioactivity measured by scintillation spectrometry.

The total intracellular histaminase content was measured in the fluid phase, obtained after incubating untreated cell suspensions with 2.5 μg/ml calcium ionophore or 0.1% Triton X-100 followed by ultrasonic disruption at 4°C (five, 5-s watt bursts separated by 1-min periods in a sonicator-cell disrupter [Heat Systems-Ultrasonics, Inc., Plainview, N. Y.]) with a microtip adaptor. Nuclear and other cell debris were removed by centrifugation at 1,000 *g* for 10 min.

Beta glucuronidase was determined on 25 μl of the reaction supernatant solution or cell lysate with phenolphthalein glucuronic acid as substrate (0.002 M final concentration) and incubated for 18 h at 37°C by the method of Talalay et al. (30).

Arylsulfatase was determined spectrophotometrically at 515 nm with *p*-nitrocatechol sulfate as substrate (5 mM) at pH 5.7 for 3 h at 37°C according to the procedure of Wasserman et al. (31).

Lactic dehydrogenase was determined with the DADE-UV-10-LDH (Dade Div., American Hospital Supply Corp., Miami, Fla.) kit by a modified Wacker method using a Perkin-Elmer Double Beam-Coleman 124 spectrophotometer at 340 nm (Perkin-Elmer Corp., Norwalk, Conn.) (32).

The total intracellular beta glucuronidase, arylsulfatase, and LDH was measured on the supernatant solution obtained after incubating untreated cell suspensions in 0.1% Triton X-100 followed by ultrasonic disruption as described above for histaminase.

RESULTS

Zymosan prepared with complement-deficient sera: effect on leukocyte enzyme release. Histaminase and β-glucuronidase release from normal neutrophils was induced by zymosan opsonized with normal human serum (Fig. 1). Zymosan opsonized with serum from patients with homozygous deficiencies of C2, C5, or C6 were equally effective in triggering release of both enzymes from neutrophils. In contrast, zymosan pre-

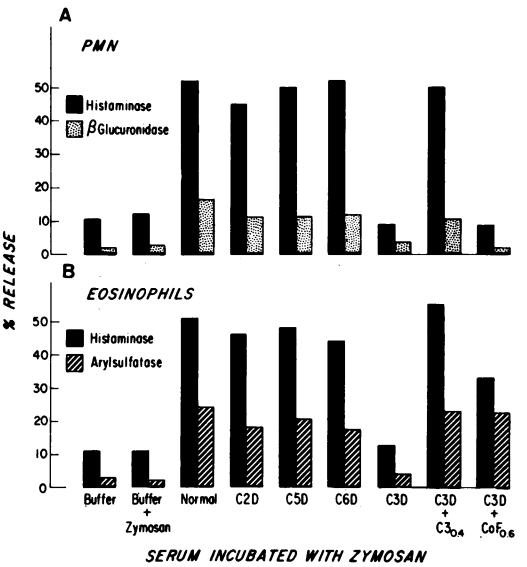

FIGURE 1 Capacity of complement-deficient sera to prepare zymosan for noncytotoxic enzyme release from human neutrophils (A) and eosinophils (B). Zymosan was washed after incubation with sera, adjusted to 2.5 particles/cell, then used to induce release. In the reconstitution experiments, radiolabeled bound C3 (0.4 μg/10^7 cells) or CoF at 0.6 μg/ml C3-deficient serum (C3D), were used. Release (average of duplicate determinations) expressed as percentage of total enzyme content (total histaminase activity, 190.5 pmol/h per 10^7 neutrophil, 208.4 pmol/h per 10^7 eosinophil; β-glucuronidase, 62.5 μg phenophthalein/h per 10^7 neutrophil; arylsulfatase, 341.7 μg nitrocatechol/h per 10^7 eosinophil). These experiments were performed more than six times and, in the case of neutrophils, 16 times with zymosan exposed to C3-deficient serum. In the latter, the mean percent histaminase release from cells exposed to zymosan prepared with normal serum was 50.9±7.4%, SD, with C3-deficient serum, 9.7±2.0% SD. Spontaneous release was 7.5±3.3% SD. D after complement stands for deficient.

pared with sera from two individuals genetically deficient in C3 (<0.01% of normal C3 concentration) failed to induce histaminase or β-glucuronidase release.

Similarly, histaminase and arylsulfatase were released from eosinophils by zymosan opsonized with normal human serum or with C2-, C5-, or C6-deficient serum, but not with C3-deficient serum. The addition of highly purified C3 or CoF to C3-deficient serum restored its ability to prepare zymosan for enzyme release from eosinophils. For neutrophils, the capacity to release histaminase by zymosan opsonized with C3-deficient serum was restored only with purified C3, but not by CoF (Fig. 1).

Effect of C3 input on histaminase release. To quantitate the effect of C3 on zymosan-induced histaminase release, varying concentrations of radiolabeled C3 were mixed with C3-deficient serum and zymosan. The opsonized zymosan was washed and a portion removed for determination of C3 uptake, and other portions used to induce enzyme release. Leukocytes were incubated with varying concentrations of a single batch of prepared zymosan particles (0.24 μg C3/particle; 0.63–5.0 particles/cell) or with batches of zymosan prepared with varying concentrations of bound C3 (2.5 particles/cell; 0.06–0.5 μg C3/particle). In each case, the extent of histaminase release from neutrophils was a function of C3 input (Fig. 2). That is, the extent of enzyme release was independent of the number of particles on which the bound C3 (C3b) was distributed. Approximately 10^5 zymosan-bound C3 molecules/cell were required to effect release of 50% of the total histaminase.

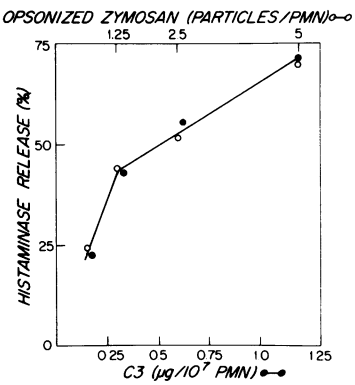

FIGURE 2 Zymosan-induced histaminase release from neutrophils as a function of particle-bound C3 concentration. Closed symbols: release induced by batches of zymosan (2.5 particles/neutrophil) prepared with varying concentrations of C3. Open symbols: release induced by a single batch of opsonized zymosan (0.24 μg C3/particle) with varying concentrations of particle/cell. Release for 1×10^7 neutrophils expressed as percentage of total enzyme content (total histaminase activity, 208.8 pmol/h per 10^7 neutrophil). PMN, polymorphonuclear leukocytes.

TABLE I
Neutrophil Histaminase Release Induced by Erythrocyte-Bound C3b

Erythrocyte complement intermediate*	C3b concentration	Histaminase release‡	
		Cells alone	Cells + CB§
	μg/10^7 erythrocytes	%	
None	0	9.1	12.2
EAC$\overline{14}$	0	11.1	14.2
EAC$\overline{1423}$	0.28	52.1	58.2
EAC$\overline{1423}$	0.40	59.9	68.3
EAC$\overline{1423}$	0.73	67.6	78.1
EAC$\overline{1423}$	1.12	79.1	90.3

* EAC$\overline{14}$ and EAC$\overline{1423}$ at 1×10^7 erythrocytes/1×10^7 neutrophils.
‡ Release expressed as percentage of total intracellular activity, 186.4 pmol/h per 10^7 neutrophils.
§ CB (5 μg/ml) preincubated with cells (20 min at 25°C).

Experiments performed in the presence of CB, at a concentration sufficient to block phagocytosis, also demonstrated that enzyme release was a function of input of bound C3/cell.

Effect of C3b bound to erythrocytes or Sephadex on enzyme release. As shown in Table I, incubation of neutrophils with EAC1423 (Sheep erythrocytes sensitized with IgM anti-Forssman antibody C1, C4, C2, and varying amounts of radiolabeled C3) resulted in dose-dependent histaminase release; release was enhanced in the presence of CB. Less than 0.28 μg of erythrocyte-bound C3 (or $\cong 1 \times 10^5$ C3b molecules/neutrophil) was required to induce 50% histaminase

TABLE II
Effect of C3b Inactivator on Induction of Neutrophil Histaminase Release by EC3b

Source of bound C3b	Erythrocyte cell number	Histaminase release*	
		Cells	Cells after C3b inactivator‡
	$\times 10^{-7}$	%	
None	0	6.3	ND‖
E§	1.0	7.2	ND
EC3b	1.25	21.5	7.1
EC3b	2.5	26.5	11.8
EC3b	5.0	40.0	13.2
EC3b	10.0	51.3	19.4

* Histaminase release as percentage of total intracellular histaminase activity, 104.7 pmol/h per 0.5×10^7 polymorphonuclear leukocytes.
‡ EC3b preincubated with purified C3b inactivator (50 μg/ml) for 20 min at 37°C.
§ Normal human erythrocytes.
‖ Not done.

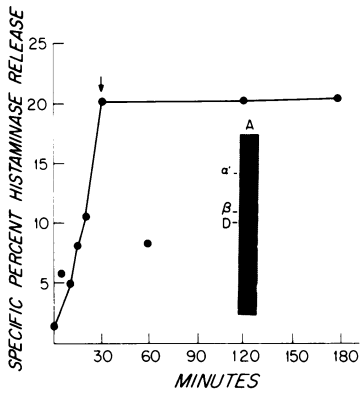

FIGURE 3 Effect of Sephadex-bound C3b on histaminase release from human polymorphonuclear leukocytes: kinetics of uptake of enzyme releasing activity. Batches of Sephadex C3b washed at timed intervals after exposure of Sephadex G-75 to normal serum (see Methods), resuspended at 1 × 10^7 beads/ml, and then incubated with neutrophils for 30 min at 37°C. Enzyme release was then assayed in the usual manner. Insert shows 1% SDS-7.5% polyacrylamide gel electrophoresis (5% mercaptoethanol) of concentrated (10 times) protein from aliquot of serum-treated, washed Sephadex G-75 then exposed to dextranase for 12 h at 25°C. The sample for SDS-polyacrylamide gel electrophoresis analysis was obtained at the time indicated by the arrow. The Coomassie Brilliant Blue-stained gel had bands corresponding to C3 a'-chain, 108,000 daltons; C3 β-chain, 70,000 daltons; and dextranase (D), 68,000 daltons. Release expressed as specific (release-spontaneous) percentage of total enzyme content (total histaminase activity, 186.3 pmol/h per 10^7 polymornuclear leukocytes.

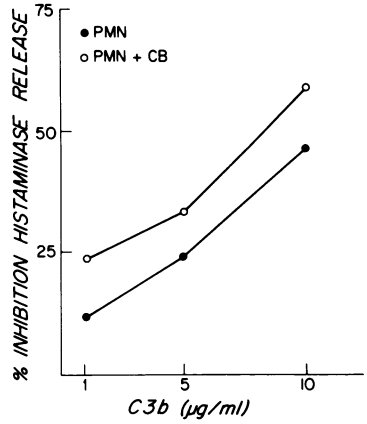

FIGURE 4 Inhibition of zymosan-induced histaminase release by fluid-phase C3b. Polymorphonuclear leukocytes (PMN) in buffer alone (●) or PMN pre-incubated with CB (○) (5 μg/ml, 20 min at 25°C). Cells incubated with fluid-phase C3b (1–10 μg/ml = 0.15–1.5 μg/0.5 × 10^7 PMN) for 20 min at 37°C, then with washed, opsonized zymosan (C3b at 0.5 μg/1.25 × 10^7 particles or 0.5 μg/0.5 × 10^7 PMN) was added and the suspension incubated for 30 min at 37°C. Results are the average of duplicate incubation mixtures. Release in the absence of fluid-phase C3b was 39.9% of total histaminase activity, 90.5 pmol/h per 0.5 × 10^7 neutrophils.

release. Human erythrocytes in the form of EC3b from a patient with genetic deficiency of C3b-inactivator, induced release of about 50% of neutrophil histaminase at a ratio of 20:1 erythrocytes:neutrophil. Decreasing numbers of erythrocytes/neutrophil lead to correspondingly less histaminase release. Release was markedly decreased by treating the erythrocytes with purified C3b-inactivator for 20 min at 37°C (Table II).

Batches of Sephadex-C3b were washed at timed intervals after incubation of Sephadex G-75 with human serum, as described in Methods. The beads were then incubated with neutrophils for 30 min at 37°C and enzyme release measured. A portion of each Sephadex-C3b batch was assayed for C3 size and structure on SDS-polyacrylamide gels and C3 protein quantitated by electroimmunodiffusion. The results, shown in Fig. 3, indicated that Sephadex-bound C3b, at a concentration of 0.15 μg/10^7 neutrophils, distributed on 3 × 10^5 Sephadex beads, induced a maximum of 20% specific histaminase release. Polyacrylamide gel electrophoresis analysis of Sephadex-bound protein (removed from the 30 min batch by dextranase) under reducing conditions showed the alpha and beta chains characteristic of C3b and a band corresponding to the size of dextranase.

Effect of fluid-phase C3b on zymosan-induced histaminase release. Neutrophils were pre-incubated with C3b for 20 min at 37°C before addition of washed zymosan that had been opsonized with normal serum. At concentrations between 1.0 and 10 μg/ml, fluid-phase C3b inhibited zymosan-induced release when the cells (5 × 10^6) were exposed to 0.5 μg bound C3b on 2.5 zymosan particles/cell (Fig. 4). Fluid-phase C3b also inhibited histaminase release induced by 1 × 10^7

TABLE III
Effect of Fluid-Phase C3b on Neutrophil Histaminase Release Induced by Erythrocyte-Bound C3b

Source of bound C3b	Erythrocyte cell number	Histaminase release*	
		Buffer	Fluid-phase C3b‡
	×10^{-7}	%	
None	0	5.2	ND‖
E§	1.0	5.4	6.4
EC3b	1.0	26.8	17.6
EC3b	2.0	35.7	23.7

* Histaminase release: percentage of total histaminase activity, 95.2 pmol/h per 0.5 × 10^7 polymorphonuclear leukocytes.

‡ Neutrophils (0.5 × 10^7) preincubated with purified fluid phase C3b (10 μg/ml or 1.5 μg/0.5 × 10^7 polymorphonuclear leukocytes) for 20 min at 37°C.

§ Normal human erythrocytes.

‖ Not done.

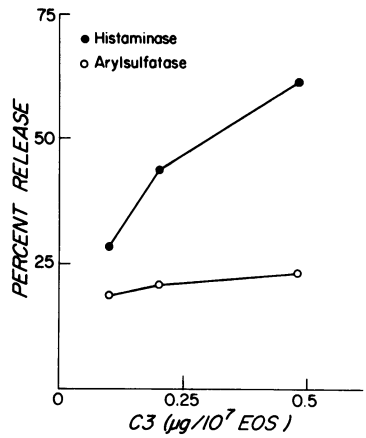

FIGURE 5 Zymosan-induced histaminase and arylsulfatase release from eosinophils (EOS) as a function of particle-bound C3b concentration. Release induced by batches of zymosan (2.5 particles/eosinophil) prepared with varying concentrations of ^{125}I-C3 (9.6×10^5 cpm/μg C3) in C3-deficient serum. Unstimulated (spontaneous) release of histaminase is 11.0% and of arylsulfatase 3.1%.

C3b-coated erythrocytes ≅50% at a concentration of fluid-phase C3b of 10 μg/ml (Table III). Fluid-phase C3b alone had no effect on enzyme release.

Zymosan-induced enzyme release from eosinophils. Eosinophil-rich cell suspensions were incubated with zymosan previously prepared with C3-deficient serum and radiolabeled C3 (Fig. 5). Particle-bound C3b at a concentration of 0.3 μg/10^7 eosinophils resulted in 50% histaminase release. In agreement with published data (13), CB-blocked, zymosan-induced enzyme release. CoF (0.6 μg/ml) in C3-deficient serum prepared zymosan for histaminase release from eosinophils (Fig. 6), but not neutrophils (Fig. 1). The amount of histaminase release was proportional to the input of CoF (0.3–0.6 μg/ml). There was no detectable uptake of radiolabeled CoF on the zymosan particles (data not shown). Zymosan incubated with C3-deficient serum; depleted of properdin factor B by immunoabsorption, did not induce histaminase or arylsulfatase release. The addition of CoF (0.6 μg/ml) alone to the C3-deficient, factor B-depleted serum did not restore its ability to prepare zymosan for enzyme release. The addition of factor B (0.12–0.3 μg/ml) and CoF to the C3-deficient, factor B-depleted serum did restore its capacity to prepare zymosan for induction of eosinophil enzyme release (Fig. 6).

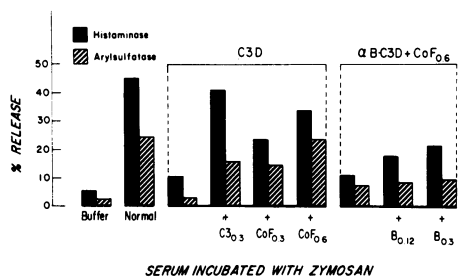

FIGURE 6 Opsonization of zymosan for noncytotoxic release of histaminase from eosinophils: capacity of CoF to substitute for C3. Zymosan incubated with C3-deficient serum (C3D) alone, with C3 (0.3 μg/10^7 cells) or with CoF at 0.3 μg/ml or 0.6 μg/ml), then washed and used at 2.5 particles/eosinophil to induce release. Right panel: other batches of zymosan prepared with C3-deficient serum depleted of factor B (as described in Methods) (α-B) with CoF at 0.6 μg/ml alone or with CoF, 0.6 μg/ml, plus purified factor B at 0.12 μg/ml or 0.3 μg/ml. In other experiments, maximal release after reconstitution of B-depleted C3-deficient serum was 34.1% for histaminase and 12.8% for arylsulfatase. Zymosan prepared with factor B-depleted, C3-deficient serum induced 7.3% histaminase and 2.1% arylsulfatase release. Total histaminase activity, 211.7 pmol/h per 10^7 eosinophils; arylsulfatase, 316.5 μg nitrocatechol/h per 10^7 eosinophils.

DISCUSSION

Several lines of evidence presented here indicate that release of histaminase and β-glucuronidase from human polymorphonuclear leukocytes can be induced by the action of particle-bound C3b at the cell surface. These include the findings that: (*a*) C3 on zymosan, erythrocytes, and Sephadex beads induced noncytotoxic enzyme release in a dose-dependent manner; (*b*) the extent of release was independent of the distribution of C3 protein on the particle (i.e., was solely dependent on the amount of C3 presented/neutrophil and not on the distribution of C3b on the particle); (*c*) enzyme release was induced even in the presence of CB at a concentration sufficient to block phagocytosis; (*d*) zymosan prepared with serum from patients genetically deficient in C3 (<0.01% normal C3 concentration) (33) failed to induce enzyme release (reconstitution of the serum required C3; CoF, a C3b analogue that is capable of activating the alternative complement pathway did not substitute for C3 for induction of enzyme release); (*e*) of the protein bound to Sephadex G-75 particles that induced release, >90% was in the form of C3b; (*f*) enzyme-releasing activity was removed from C3b-coated erythrocytes by the action of highly purified C3b inactivator; and (*g*) zymosan-and EC3b-induced release was inhibited by fluid-phase C3b. Relatively low concentrations of fluid-phase C3b were required, because it is probable that only a small portion of the particle-bound C3b is presented to the cells.

The calculated requirement of about 10^5 particle-bound C3b molecules/neutrophil to effect 50% histaminase release must be considered maximum; a minimum estimate is not possible under these experimental conditions. Only a few hundred C3b molecules/cell are required to generate the immune adherence phenomenon (34) or immune hemolysis (35).

The capacity of C5a to induce β-glucuronidase release is well-established (10), but its effect on histaminase release had not yet been tested. However, in the present studies, an absolute requirement for particle-bound C5b, the larger fragment derived from activation of C5, was excluded by the finding that release induced by zymosan prepared in C5-deficient serum was indistinguishable from normal.

Results of an earlier study (12) suggested a qualitative difference in mechanism of release of histaminase from eosinophils and neutrophils. CB, at concentrations that block phagocytosis and enhance enzyme release from neutrophils, inhibited zymosan-induced histaminase and arylsulfatase release from eosinophils (12). The observation that CoF, a C3b analogue, can substitute for C3 itself in preparing zymosan for induction of enzyme release from eosinophils, but not neutrophils, revealed another qualitative difference between the two cell types with respect to release mechanisms, i.e., an absolute dependence on bound C3b in the neutrophil, but not for the eosinophil. There was no detectable uptake of CoF on the zymosan. It is probable that "opsonization" was accomplished by deposition on zymosan of a serum component other than C3b that depends on activation of the alternative pathway. Support for the requirement of an otherwise intact alternative pathway for enzyme release from eosinophils was obtained in an experiment, where depleting factor B in a C3-deficient serum blocked the ability of CoF to reconstitute the serum. Addition of purified factor B back to the mixture generated an activity on zymosan that promoted phagocytosis (data not shown) and noncytotoxic enzyme release. These data are consistent with those of Metcalf et al. (36) who showed a requirement for alternative complement pathway activity for particle-bound arylsulfatase release from human eosinophils. However, our findings indicate that in the absence of C3, deposition of other properdin pathway-dependent proteins can prepare a particle for induction of histaminase and arylsulfatase release from eosinophils.

The data presented here and elsewhere (31) suggest that local tissue concentrations of allergic mediators, i.e., histamine and slow reacting substance of anaphylaxis may be regulated by complex-reciprocating control mechanisms. For example, cleavage of C3 to C3a and C3b would effect release of histamine from mast cells (C3a) and histaminase from neutrophils or eosinophils (C3b). The rate of generation of C3 cleavage products, their rates of inactivation by anaphylatoxin inactivator and the C3b inactivator (11) (including β_1H), and the kinetics of histaminase and arylsulfatase activity may serve to modulate the biological effects of histamine and slow reacting substance of anaphylaxis.

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