Inability of Rat Anaphylatoxin to Induce Histamine Release in Rats

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Abstract—The role of rat anaphylatoxin in histamine release and increased vascular permeability during the first thirty minute period in zymosan-air-pouch inflammation, an experimental model of inflammation induced by zymosan in an air-pouch prepared on the back of rats, was investigated. Complement depletion by cobra venom factor did not affect the histamine release nor the increased vascular permeability in the inflammation of this type. In spite of apparent anaphylatoxin activity, zymosan activated serum (ZAS) failed to cause any significant release of histamine when infused in the air-pouch on the back. Anaphylatoxin purified from rat serum activated with zymosan in the presence of an inhibitor (-aminocaproic acid) of anaphylatoxin inactivator gave a single band in both polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE. The molecular weight estimated by SDS-PAGE was approx. 7,000. The purified rat anaphylatoxin failed to induce histamine release nor increased vascular permeability even at 50 ng/ml, although it caused contraction of guinea pig ileum at 0.8 ng/ml. These results suggest that rat anaphylatoxin does not participate in histamine release and increased vascular permeability in the zymosan-air-pouch inflammation.

Zymosan-air-pouch inflammation, an experimental model of inflammation of the air-pouch type induced on the back of rats with the aid of zymosan, was introduced in this laboratory as a novel experiment model of inflammation (1), which is characterized by an intense migration of polymorphnuclear leukocytes into the pouch fluid, marked aggregation of the leukocytes on the inner surface of the inflammatory pouch and the formation of capsular granulation tissues as well as accumulation of exudate fluid in the pouch. Activation of the alternative pathway of the complement system by zymosan has been assumed to be involved in the pathogenesis of the zymosan-air-pouch inflammation (1).

Activation of the complement system is known to cause generation of anaphylatoxins such as C3a, C4a and C5a (2-5) and then to induce mast cell degranulation (2, 6, 7). The mast cell degranulation is immediately followed by the release of vasoactive amines, histamine and serotonin from the granules liberated (6, 7). Consequently, the degranulation is generally accompanied with elevation of vascular permeability in the local tissues (8-10).

In the present study, we examined whether or not rat anaphylatoxin participates in the histamine release and vascular permeability response observed during the first thirty minute period in the zymosan-air-pouch inflammation.

Materials and Methods

Animals: Male Sprague-Dawley rats, specific pathogen free, purchased from Charles River Japan, Inc., Kanagawa, Japan and male Hartley guinea pigs from Funabashi Farm, Chiba, Japan were used.

Induction of zymosan-air-pouch inflammation: The zymosan-air-pouch inflammation was induced as described (1). In brief, rats (150-210 g body weight) were injected with 8 ml of air subcutaneously on the back under
light ether anaesthesia to make an ellipsoid or oval air-pouch. Twenty-four hours later, 4.0 ml of 1.6% (w/v) zymosan (zymosan A, Sigma Chemical Co., St. Louis, MO, U.S.A.) suspension in 0.8% sodium carboxymethyl cellulose (CMC-Na, Cellogen F-3H, Dai-ichi Kogyo Seiyaku Co., Kyoto, Japan) solution in saline, supplemented with antibiotics (0.1 mg penicillin G potassium and 0.1 mg dihydrostreptomycin sulfate per 1.0 ml), were injected into the preformed air-pouch to provoke an inflammatory response.

Serum anaphylatoxin-induced inflammation: Rats were injected into the preformed air-pouch with 4 ml of 0.8% CMC solution containing zymosan activated serum (ZAS) at 20% by volume. For the preparation of ZAS, 10 mg of zymosan were added to 1.0 ml of normal rat serum containing an inhibitor of serum carboxypeptidase N (CPNI, D,L-2-mercaptomethyl-3-guanidinoethyl thiopropanoic acid, Carbiochem-Behring Co., La Jolla, CA, U.S.A.) at 1 mM. The mixture was incubated at 37°C for 30 min and then centrifuged at 3,000 x g for 10 min at 4°C to remove zymosan. The supernatant was heated at 56°C for 30 min. Non-activated serum (NAS) was prepared by heating normal rat serum containing 1 mM CPNI at 56°C for 30 min without zymosan and used in place of ZAS as the control.

Complement depletion: The complement depletion study was done by the use of purified cobra venom factor (CVF, Cordis Laboratories, Inc., Miami, FL U.S.A.) which was derived from Naja naja without contamination of lecithinase (phospholipase A2). The depletion of complement in vivo was brought about according to the method of Cochrane et al. (11). In brief, rats received intraperitoneal injections consisting of four equal doses (62.5 units/kg body weight, 4 times) at 0, 14, 20 and 24 hr prior to making the air-pouch. For the determination of complement-inhibitory capacity of the CVF, 0.1 ml of serial dilutions of the purchased CVF was incubated firstly with 0.4 ml of a 1:20 dilution of normal human serum (NHS) at 37°C for 20 min and followed by 60 min incubation with 5×10⁸ sensitized sheep erythrocytes (EA) under gentle shaking. Veronal buffer supplemented with CaCl₂ and MgCl₂ (final concentrations of 0.15 mM and 0.5 mM, respectively) was used as the diluent (VB⁺⁺). At the termination of the incubation, 2 ml of cold VB⁺⁺ were added and centrifuged to remove unlysed erythrocytes. The optical absorbency of the supernatants were then measured at 541 nm. One unit of CVF is defined as the quantity of CVF in 0.1 ml that reduces the hemolytic capacity of 0.4 ml of the 1:20 dilution of NHS by 50%.

Measurement of CH50: Serum CH50 was assayed according to the method of Mayer (12) with a modification (13) in which the volume of samples and all the reagents used was reduced to 1/5 of those in the original method.

Measurement of plasma exudation: Plasma exudation was measured with the aid of fluorescein-labeled bovine serum albumin (F-BSA) as a tracer (14). The amount of exuded F-BSA into the pouch fluid during the thirty minute period after the intravenous injection of F-BSA (20 mg/0.2 ml of saline) was measured by reading its fluorescence intensity at 521 nm under the excitation at 490 nm. The plasma exudation in the entire volume of the pouch fluid was calculated and expressed as percent of F-BSA injected.

Measurement of histamine content in the pouch fluid: The pouch fluid collected was diluted twice with ice-cold phosphate-buffered saline (PBS) at pH 7.4 and then centrifuged at 2,000 x g for 20 min at 4°C. One ml of the supernatant was mixed with 2.0 ml of 0.4 N HClO₄, and the mixture was centrifuged at 2,000 x g for 30 min at 4°C in order to remove protein. Histamine content in the supernatant was assayed by the method of von Redlich and Glick (15) with a modification using reagent and sample at 10 times the volumes used in the original method.

Measurement of protein content: Protein content was determined according to Lowry's method (16) using bovine serum albumin as a reference standard.

Anaphylatoxin assay: Anaphylatoxin activity was tested with terminal strips of guinea pig ileum as described by Wissler (17).

Anaphylatoxin purification: Rat anaphylatoxin was purified according to the method described by Fernandez and Hugli (18), Jose et al. (19) and Hugli et al. (4).
Three liters of rat serum were activated by incubating with zymosan (5 g/liter, 37°C for 60 min) in the presence of 1 M ε-amino-n-caproic acid (EACA), an inhibitor of serum carboxypeptidase N, and then centrifuged to remove the zymosan. The supernatant was then heated at 58°C for 60 min to inactivate carboxypeptidase and then cooled to 20°C. The pH was adjusted to 4.3 by the addition of 1M HCl. Ethylenediaminetetraacetic acid disodium (EDTA) was then added to a final concentration of 10 mM and the mixture was centrifuged. All subsequent operations were carried out at 4°C. The supernatant was dialyzed in small-pore tubing (Spectrapor 3, M.W. cutoff, approx. 3,600; Spectrum Medical Industries, Inc., Los Angeles) against 5 changes of the dialysis buffer (0.1 M ammonium formate, pH 5.0, containing 0.5 mM EDTA) to reduce the concentration of EACA to the level of not more than 1 mM and then centrifuged and passed through a column (2.8x75 cm) of CM-cellulose (fibrous form; James River, Co., Berlin, NH, U.S.A.) equilibrated in the dialysis buffer. The column was washed with 1 liter of the dialysis buffer, and then anaphylatoxin was eluted with 1.5 liters of 0.5 M ammonium formate, pH 5.0.

The addition of an equal volume of ethanol to the eluate precipitated a large amount of protein, which was removed by centrifugation. The supernatant was evaporated in a rotary evaporator to reduce the volume, dialyzed against water, lyophilized, dissolved in 0.1 M ammonium formate, pH 5.0, and chromatographed on Sephadex G-100 in the same buffer (a column: 3.0X94 cm). Fractions containing anaphylatoxin activity were pooled and passed through a column (2.0X18 cm) of SP-Sephadex C-25 previously equilibrated in the same buffer. The column was washed with 0.1 M ammonium formate, pH 7.0 (150 ml). The anaphylatoxin was eluted from the column by the same method as described above. Fractions containing anaphylatoxin activity were pooled, dialyzed against water and lyophilized.

Polyacrylamide gel electrophoresis (PAGE): Disc electrophoresis was carried out in 15% polyacrylamide gels at pH 4.3 according to Reisfeld et al. (20). Gels were stained with Coomassie brilliant blue G250 (Fluka AG, Chem. Fabrik CH-9470 Buchs) (21).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE was carried out according to the method of Swank and Munkres (22). The molecular weight of anaphylatoxin was estimated by using the polypeptide molecular weight calibration kit purchased from Pharmacia Fine Chemicals (Pharmacia Fine Chemicals, New Jersey, U.S.A.) as markers.

Skin reaction: A 0.1 ml aliquot of the test sample was injected intradermally into the clipped back of a rat immediately after intravenously injecting 25 mg/kg body weight of Evans blue (2.5% solution in 0.6% saline). Vascular permeability increasing activity of the sample was determined at 30 min after its intradermal injection by measuring the amount of extravasated Evans blue according to the dye extraction method of Udaka et al. (23). The activity was expressed as micrograms of the dye.

Results

Effect of complement depletion by cobra venom factor (CVF) on zymosan-air-pouch inflammation: Serum complement level in rats pretreated with CVF (62.5 units/kg x 4) were reduced to 0.2% of the control saline treated level in rats (Fig. 1). However, the plasma exudation and histamine release in the first thirty minute period in the zymosan air-pouch inflammation were not inhibited by the complement depletion.

Failure of zymosan activated serum (ZAS) to induce histamine release: In order to examine whether or not ZAS having anaphylatoxin activity may induce histamine release from rat connective tissue mast cells, 0.8% CMC solution containing ZAS at 20% by volume or non-activated serum (NAS) without anaphylatoxin activity was injected into the preformed air-pouch. Histamine
level in the pouch fluid was measured 30 min after. The histamine level in the pouch treated with ZAS was 86±19 ng/ml (n=6). This is very markedly lower than the case of the zymosan-air-pouch inflammation, i.e., 390 ng/ml as shown in Fig. 1, and very close to the histamine level (39±5 ng/ml, n=6) in the pouch treated with NAS as well as to the usual level of histamine (30-60 ng/ml) in the pouch treated with the vehicle, 0.8% CMC solution, only.

**Purification of rat anaphylatoxin:** Rat anaphylatoxin was isolated from zymosan activated serum in order to examine its pharmacological properties. Anaphylatoxin generated in rat serum through activation with zymosan in the presence of an inhibitor of serum carboxypeptidase N, EACA, was purified by chromatography with CM-cellulose, Sephadex G-100 and SP-Sephadex C-25. Figures 2 to 4 depict the elution behavior of the anaphylatoxin at each chromatographic step of the purification procedure. Anaphylatoxin was eluted as one symmetrical peak from SP-Sephadex C-25 and other fractions did not show any anaphylatoxin activity. No other anaphylatoxin activity was recovered from the CM-cellulose column by elution with 1 M ammonium formate buffer after the elution with 0.5 M ammonium formate buffer at pH 5.0. Fractions (4.6 ml/fraction) were collected and assayed for anaphylatoxin activity, and then fractions indicated by the bar were pooled. Anaphylatoxin activity is represented by the symbol +.
activated serum was retained by the resin during the CM-cellulose adsorption.

The purity of the rat anaphylatoxin was appraised by polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE. In PAGE, gels heavily loaded with 80 μg of the final material showed a single band (Fig. 5A). In SDS-PAGE also, gels containing 12 μg of the final material showed a single band (Fig. 5B), and the band was unaffected by reduction with 2-mercaptoethanol (data not shown). The molecular weight of the purified rat anaphylatoxin was estimated by means of SDS-PAGE, using a polypeptide molecular weight calibration kit (Pharmacia Fine Chemicals) as markers, to be approximately 7,000 (Figs. 5B and 5C). The minimal concentration of the purified rat anaphylatoxin necessary to evoke guinea pig ileum contraction was 0.8 μg/ml (1.1×10⁻⁷ M).

Failure of rat purified anaphylatoxin to induce histamine release: The purified rat anaphylatoxin (50 μg/ml) dissolved in 0.8% CMC solution was injected into the air-pouch preformed on the back of rats. In this experiment, CMC was dissolved in Hank’s balanced salt solution, pH 7.4, to prevent anaphylatoxin from binding with CMC. Neither histamine release nor vascular permeability response was observed in the first thirty minute period (Fig. 6).

When tested by intradermal injection, the purified rat anaphylatoxin dissolved in Tyrode solution (pH 7.4) failed to induce vascular permeability response, although 0.2 μg serotonin used as a reference evoked a strong reaction (Fig. 7).

Discussion

We reported in a previous paper that a novel anticomplementary agent, K-76COONa, inhibited leukocyte migration in the inflammatory sites in zymosan-air-pouch inflammation (1). Therefore, generation of C3a and C5a, proinflammatory fragments of the complement, via activation of the alternative pathway of the complement system by zymosan was assumed to play an important role in induction of the inflammatory responses.

The present experiments were undertaken to gain further insight into mechanisms of zymosan-induced inflammation, especially to examine whether or not rat anaphylatoxin participates in the processes for histamine release and plasma exudation which were observed, as shown in Fig. 1, in the first thirty minute period. Consumption of the complement in the pouch was also confirmed (data not shown here, but will be reported elsewhere). These observations appeared to indicate that rat anaphylatoxin generated via complement activation by zymosan would have participated in the inflammatory responses induced with zymosan. On the other hand, however, the finding that the complement depletion brought about by the pretreatment of rats with CVF failed to inhibit histamine release and plasma exudation (Fig. 1) suggested that rat anaphylatoxin might not have participated in the inflammatory responses induced with zymosan. Moreover, rat serum anaphylatoxin (ZAS) and the purified anaphylatoxin failed to induce histamine release from rat connective tissue mast cells (Fig. 6). These results are consistent with observations that rat serum anaphylatoxin injected intra-
Fig. 5. Disk electrophoresis of rat purified anaphylatoxin. A. Polyacrylamide gel electrophoresis (PAGE). Left: 80 μg of rat purified anaphylatoxin applied. Right: 30 μg of rat purified anaphylatoxin applied. B. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Left: 12 μg of rat purified anaphylatoxin applied. Center: 12 μg of rat purified anaphylatoxin and 17 μg of standard peptides applied. Right: 17 μg of standard peptides applied. The molecular weights of standard peptides were represented near their bands. These gels were stained for protein by Coomassie brilliant blue G250. C. Determination of molecular weight of rat purified anaphylatoxin by the SDS-PAGE. The molecular weights of standard peptides are shown beside near the points (solid circles) which indicate their relative mobilities. The open circle indicates the relative mobility of purified rat anaphylatoxin. Relative mobility is defined as:

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\text{Relative mobility} = \frac{\text{distance polypeptide has migrated from origin}}{\text{distance from origin to reference point}}.
\]

The position of the tracking dye bromophenol blue was used as the reference point.

Venously into a rat was unable to disrupt rat skin mast cells nor to increase plasma histamine (24). It is also incapable of disrupting rat mesentery mast cells, although guinea pig mesentery mast cells can be degranulated (24). Regal et al. also reported that partially purified guinea pig C5a failed to induce not only increased vascular permeability in the rat skin but also histamine release from rat lung at concentrations sufficient to cause mast cell degranulation and histamine release in the skin and lung.
of guinea pigs. (25). On the other hand, Johnson et al. (7) reported that porcine C5a was capable of inducing histamine release from rat peritoneal cells, although the concentration (10^{-6}-10^{-5} M) was very much higher than its minimal effective concentration to induce contraction of guinea pig ileum (1.8\times10^{-10} M) (26). In the present experiments, we were unable to demonstrate vascular permeability response in the rat skin with the purified rat anaphylatoxin at the concentration of 1.4\times10^{-8} M, although the minimal effective concentration to contract guinea pig ileum was 1.1\times10^{-7} M (Fig. 7).

Human anaphylatoxins, C3a and C5a, are known to be inactivated by carboxypeptidase N (anaphylatoxin inactivator) which exists not only in human serum but also in sera from various animal species such as rats, guinea pigs and rabbits (27). In contrast, the anaphylatoxins of rat and guinea pig serum are resistant to the inactivation by autologous and heterologous anaphylatoxin inactivator (27). Furthermore, Gerard and Hugli reported that human des-Arg^{74}-C5a exhibited a potency which was only 0.1 percent that of intact human C5a, while porcine des-Arg^{74}-C5a possessed approximately 30 percent of the activity of intact porcine C5a (26). Based on these findings, they concluded that the stability of nonhuman anaphylatoxins was attributable to nonessentiality for anaphylatoxin activity of the arginyl residue at the carboxy terminal of nonhuman anaphylatoxins (26). Therefore, it is unlikely that the inability of rat anaphylatoxin to induce histamine release from rat connective tissue mast cells is due to its hydrolytic inactivation by the anaphylatoxin inactivator.

Up to now, there has been only one report (17) dealing with the purification of rat anaphylatoxin, probably because of the difficulty in collecting a large volume of blood from rats. The purification procedure reported (17) is very laborious, involving eight complex steps. In the present study, the purification procedure of rat anaphylatoxin was improved and simplified, consisting of only four steps. The purified anaphylatoxin with our method showed a single band both in PAGE and in SDS-PAGE (Fig. 5A and 5B), and the minimal concentration to contract guinea pig ileum was 1.1\times10^{-7} M, which was similar to that of rat anaphylatoxin (1.7\times10^{-7} M) previously reported (17, 28).

In conclusion, the present study has revealed that rat anaphylatoxin may not participate in histamine release and plasma exudation observed in the first thirty minute period in the zymosan-induced inflammation.

Fig. 6. Inability of rat purified anaphylatoxin to cause histamine release or to induce of plasma exudation in the air-pouch. Thirty minutes after the injection into the preformed air-pouch with 0.8% CMC solution containing 50 \mu g/ml of rat purified anaphylatoxin, the amount of histamine and plasma exudation in the pouch fluid were determined. Figures in the columns indicate the number of rats used. Columns represent the means and vertical bars show the S.E.M.

Fig. 7. Inability of rat purified anaphylatoxin to induce plasma leakage in the rat skin. Dye leakage in the skin injected intradermally with 0.1 ml solutions of rat purified anaphylatoxin and 5-HT was measured as described in the text. Columns represent the mean of five to seven rats and vertical bars show the S.E.M.
since (1) rat serum anaphylatoxin and purified rat anaphylatoxin failed to induce histamine release in rat connective tissues and (2) the complement depletion with CVF failed to inhibit plasma exudation and histamine release in the zymosan-air-pouch inflammation.

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References
1 Konno, S. and Tsurufuji, S.: Induction of zymosan-air-pouch inflammation in rats and its characterization with reference to the effects on anticomplementary and anti-inflammatory agents. Br. J. Pharmacol. 80, 269–277 (1983)
2 Dias da Silva, W. and Lepow, I.H.: Complement as a mediator of inflammation. II. Biological properties of anaphylatoxin prepared with purified components of human complement. J. Exp. Med. 125, 921–946 (1967)
3 Cochrane, C.G. and Müller-Eberhard, H.J.: The derivation of two distinct anaphylatoxin activities from the third and fifth components of human complement. J. Exp. Med. 127, 371–386 (1968)
4 Hugli, T.E., Gerard, C., Kawahara, M., Scheetz II, M.E., Barton, R., Briggs, G., Koppel, G. and Russell, S.: Isolation of three anaphylatoxins from complement-activated human serum. Mol. Cell. Biochem. 41, 59–66 (1981)
5 Hugli, T.E. and Müller-Eberhard, H.J.: C3a and C5a. Adv. Immunol. 26, 1–53 (1978)
6 Mota, I.: The mechanism of action of anaphylatoxin. Its effect on guinea pig mast cell. Immunology, 2, 403–413 (1959)
7 Johnson, A.R., Hugli, T.E. and Müller-Eberhard, H.J.: Release of histamine from rat mast cells by the complement peptides C3a and C5a. Immunology 28, 1067–1080 (1975)
8 Lepow, I.H., Willms-Kretschmer, K., Parrock, R.A. and Rosen, F.S.: Gross and ultrastructural observations on lesions produced by intradermal injection of human C3a in man. Am. J. Pathol. 61, 13–23 (1970)
9 Wuepper, K.D., Bokisch, V.A., Müller-Eberhard, H.J. and Stoughton, R.B.: Cutaneous responses to human C3 anaphylatoxin in man. Clin. Exp. Immunol. 11, 13–20 (1972)
10 Damerau, B. and Vogt, W.: Effect of hog anaphylatoxin (C6a) on vascular permeability and leukocyte emigration in vivo. Naunyn Schmiedebergs Arch. Pharmacol. 295, 237–241 (1976)
11 Cochrane, C.G., Müller-Eberhard, H.J. and Akin, B.S.: Depletion of plasma complement in vivo by a protein of cobra venom: its effect on various immunologic reactions. J. Immunol. 105, 55–69 (1970)
12 Mayer, M.M.: Complement and complement fixation. In Experimental Immunochemistry (2nd ed.), Edited by Kabat, E.A. and Mayer, M.M., p. 133–240, Charles C. Thomas, Springfield (1961)
13 Okada, H.: Complement assay methods for clinical laboratories. Rinshó Men-eki 5, 951–363 (1973) (in Japanese)
14 Watanabe, K., Nakagawa, H. and Tsurufuji, S.: A new sensitive fluorometric method for measurement of vascular permeability. J. Pharmacol. Method 11, 167–178 (1984)
15 von Redlich, D. and Glick, D.: Studies in histochemistry. LXXVI. Florometric determination of histamine in microgram samples of tissue or microliter volumes of body fluids. Anal. Biochem. 10, 469–467 (1967)
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 285–275 (1951)
17 Wissler, J.H.: Chemistry and biology of the anaphylatoxin related serum peptide system. I. Purification, crystallization and properties of classical anaphylatoxin from rat serum. Eur. J. Immunol. 2, 73–83 (1972)
18 Fernandez, H.N. and Hugli, T.E.: Partial characterization of human C5a anaphylatoxin. I. Chemical description of the carbohydrate and polypeptide portions of human C5a. J. Immunol. 117, 1688–1694 (1976)
19 José, P.J., Forrest, M.J. and Williams, T.J.: Human C5a des Arg increases vascular permeability. J. Immunol. 127, 2376–2380 (1981)
20 Reisfeld, R.A., Lewis, U.J. and Williams, D.E.: Disk electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature 195, 281–283 (1962)
21 Blakesley, R.W. and Boezi, J.A.: A new staining technique for proteins in polyacrylamide gels using Coomassie brilliant blue G250. Anal. Biochem. 82, 580–582 (1977)
22 Swank, R.T. and Munkres, K.D.: Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. Anal. Biochem. 39, 462–477 (1971)
23 Ueda, K., Takeuchi, Y. and Movat, H.Z.: Simple method for quantitation of enhanced vascular permeability. Proc. Soc. Exp. Biol. Med. 133, 1384–1387 (1970)
24 Mota, I.: Action of anaphylactic shock and
anaphylatoxin on mast cells and histamine in rats. Br. J. Pharmacol. 12, 453-456 (1957)

25 Regal, I.F., Hardy, T.M., Casey, F.B. and Chakrin, L.W.: C5a-induced histamine release. Species specificity. Int. Arch. Allergy Appl. Immunol. 72, 362-365 (1983)

26 Gerard, C. and Hugli, T.E.: Identification of classical anaphylatoxin as the des-Arg form of the C5a molecule: Evidence of a modulator role for the oligosaccharide unit in human des-Arg74-C5a. Proc. Natl. Acad. Sci. U.S.A. 78, 1833-1837 (1981)

27 Bokisch, V.A. and Müller-Eberhard, H.J.: Anaphylatoxin inactivator of human plasma: Its isolation and characterization as a carboxypeptidase. J. Clin. Invest. 49, 2427-2436 (1970)

28 Wissler, J.H., Stecher, V.J. and Sorkin, E.: Chemistry and biology of the anaphylatoxin related serum peptide system III. Evaluation leucotactic activity as a property of new peptide system with classical anaphylatoxin and corytotoxin as components. Fur. J. Immunol. 2, 90-96 (1972)