Molecular Immunology Vol. 20, No. 4, pp. 377–382, 1983
Printed in Great Britain.

0161-5890/83/040377-06$03.00/0

THE MEMBRANE ATTACK COMPLEX OF COMPLEMENT AND ITS PRECURSOR PROTEINS LACK PHOSPHOLIPASE ACTIVITY*

CARL-WILHELM VOGEL,†‖ ANDREAS PLÜCKTHUN,‡¶ ECKHARD R. PODACK,†
EDWARD A. DENNIS‡ and HANS J. MÜLLER-EBERHARD†§

†Department of Molecular Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037, U.S.A. and
‡Department of Chemistry, University of California at San Diego, La Jolla, California 92093, U.S.A.

(*Received* 31 *August* 1982; *accepted* 20 *October* 1982)

Abstract—The membrane attack complex of human complement and its highly purified precursor proteins have been analyzed for phospholipase activity. Using three different sensitive assays, phospholipase A_1, A_2, C or D activity could not be detected. Based on the sensitivity of the assays employed, these results indicate the complement-mediated membrane damage is not enhanced by covalent breakdown of membrane phospholipids, but is entirely caused by physical action of the membrane attack complex. The results also imply that the putative serine esterase sites of C6 and C7 are not acting on phospholipids.

INTRODUCTION

The membranolytic activity of complement is believed to be nonenzymatic and entirely due to physical interaction of the membrane attack complex (MAC)** with the target membrane (Esser *et al.*, 1979; Mayer, 1978; Podack *et al.*, 1982*a*). Recently, however, it has been shown that the hemolytic activities of C6 and C7 can be inhibited by the serine esterase inhibitors DFP, TLCK, PMSF, and pAPMSF (Kolb *et al.*, 1982; Kolb & Kolb, 1982). These observations necessitate the search for the physiological substrates of these active sites.

A possible substrate is the phospholipid of the target membrane since not only the serine proteases but also the lipases are known to be serine esterases, hydrolyzing their substrates by the well characterized double intermediate reaction mechanism (Brockerhoff, 1973; Brockerhoff, 1974). Lipases have also been shown to exhibit phospholipase A_1 activities†† (Slotboom *et al.*, 1970; Fischer *et al.*, 1973; deHaas *et al.*, 1965). While phospholipases A_2 secreted from exocrine glands do not follow the serine esterase mechanism (Dijkstra *et al.*, 1981; Kamer & Argos, 1981) very little is known about intracellular or serum phospholipases. These results prompted us to reinvestigate an old question of complement action: the possible enzymatic activity of the MAC on phospholipids.

It has been clearly established that complement-mediated membranolysis is not accompanied by extensive cleavage of membrane phospholipids (Humphrey & Dourmashkin, 1965; Inoue & Kinsky, 1970; Hesketh *et al.*, 1972; Kinoshita *et al.*, 1977; Smith & Becker, 1968; Lachmann *et al.*, 1973; Kinsky *et al.*, 1971; Inoue *et al.*, 1974*a*; Inoue *et al.*, 1974*b*). While some investigators could not detect any phospholipid cleavage products (Humphrey &

* This is publication number 2693 from the Research Institute of Scripps Clinic. This investigation was supported by United States Public Health Service Grants AI 17354, CA 27489, GM 20501, HL 07195 and HL 16411. C.-W.V. is recipient of Deutsche Forschungsgemeinschaft Fellowship No. Vo 303/1. A.P. is recipient of a Fellowship from Studienstiftung des Deutschen Volkes. E.R.P. is an Established Investigator of the American Heart Association, No. 79-149. H.J.M.-E. is the Cecil H. and Ida M. Green Investigator in Medical Research, Research Institute of Scripps Clinic.

§ Author to whom correspondence should be sent: Dr. Hans J. Müller-Eberhard, Research Institute of Scripps Clinic, Department of Immunology, 10666 North Torrey Pines Road, La Jolla, California 92037, U.S.A.

‖ Current address: Department of Biochemistry, Georgetown University School of Medicine, 3900 Reservoir Road, N.W., Washington, D.C. 20007, U.S.A.

¶ Current address: Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, U.S.A.

** Abbreviations: MAC, membrane attack complex; poly C9, polymerized C9; DFP, diisopropylfluorophosphate; TLCK, *p*-tosyl-L-lysinechloromethylketone; PMSF, phenylmethylsulfonylfluoride; pAPMSF, *p*-amidinophenylmethanesulfonylfluoride; TLC, thin layer chromatography; FID, flame ionization detection.

†† Enzymatic activities mentioned in this paper: phospholipase A_1 (EC 3.1.1.32), phospholipase A_2 (EC 3.1.1.4), phospholipase C (EC 3.1.4.3), phospholipase D (EC 2.1.4.4), tributyrinase = triacylglycerol lipase (EC 3.1.1.3), phenyl laurate esterase = acylesterase (EC 3.1.1.2).

Dourmashkin, 1965; Inoue & Kinsky, 1970; Hesketh *et al.*, 1972; Kinoshita *et al.*, 1977), other investigators found phospholipid cleavage products, but only small quantities (Smith & Becker, 1968; Lachmann *et al.*, 1973). Liposomes prepared from a phospholipid analog resistant to phospholipase A_1, A_2, C and D were susceptible to complement-mediated lysis, though to a lesser extent than appropriate controls (Kinsky *et al.*, 1971). In the case of a bacterial target the formation of phospholipid cleavage products has been shown to be predominantly a result of a bacteria-derived phospholipase A_2 (Inoue *et al.*, 1974*a*; Inoue *et al.*, 1974*b*). These results taken together indicate that enzymatic action on membrane lipids is neither essential for membranolysis nor extensive. Nevertheless, the possibility remained that enzymatic sites on one or more of the MAC precursor proteins are present, enhancing the physical effect of the MAC by a low degree of phospholipid hydrolysis. This concept has been suggested (Mayer, 1972) and, in addition to the above mentioned serine esterase sites in C6 and C7, several enzymatic activities have been described for MAC precursor proteins. A phenyl laurate esterase (Delâge *et al.*, 1969; Simard *et al.*, 1969) and tributyrinase (Delâge *et al.*, 1973) activity have been described for C7, the former activity possibly also present in C6 (Delâge *et al.*, 1969). Phospholipase A_2 activity has been suggested to be associated with C8 (Okada & Campbell, 1974; Scherer *et al.*, 1976).

In all studies mentioned whole serum or partially purified complement components were used. As pointed out in a theoretical paper (Mayer, 1972) highly purified complement proteins will have to be used to finally answer the question of enzymatic activity associated with the MAC action. In an attempt to solve this problem we subjected the highly purified complement proteins C5, C5b–6, C6, C7, C8, C9, the recently described poly C9 (Podack & Tschopp, 1982*a*; Podack *et al.*, 1982*b*; Tschopp *et al.*, 1982; Podack & Tschopp, 1982*b*) and the forming MAC to three sensitive assays for phospholipase activity.

MATERIALS AND METHODS

Human complement components C5 and C9 were purified as described (Hammer *et al.*, 1981) with the following modifications. For the C5 purification the gel filtration was omitted. The C5 pool from the hydroxylapatite column was dialyzed vs 3.5 m*M* 5,5′-diethylbarbituric acid (Sigma), 143 m*M* NaCl, pH 7.4 and applied to a 20 ml Concanavalin A–Sepharose CL4B (Pharmacia) column. C5 was eluted with 0.2 *M* α-methyl-mannoside (Sigma). The final step for C9 purification was hydroxylapatite (Bio-Rad) chromatography as described (Biesecker & Müller-Eberhard, 1980). C5b–6 (Podack & Müller-Eberhard, 1980), C6 (Podack *et al.*, 1979), C7 (Podack *et al.*, 1979) and C8 (Kolb & Müller-Eberhard, 1976) were isolated from fresh serum or plasma (San Diego Blood Bank). Polymeric C9 (poly C9) was prepared as described (Podack *et al.*, 1982*b*). Phospholipase A_2 was purified from lyophilized cobra venom (*Naja naja naja*) (Miami Serpentarium) (Deems & Dennis, 1980; Darke *et al.*, 1980). Phosphatidylcholine was prepared from egg yolk (Singleton *et al.*, 1965).

Phospholipase activity was determined by three different methods: (a) with a flame ionization detector after thin layer chromatography of reaction products on silica gel coated quartz rods (chromatorods) (TLC/FID assay) (Ackman, 1981); (b) with a hemolytic assay based on the synergistic action with cardiotoxin (Vogel *et al.*, 1981), and (c) by using radiolabeled substrate and the subsequent separation of reaction products by thin layer chromatography (Grossman *et al.*, 1974; Kensil & Dennis, 1979). In all three assays purified phospholipase A_2 from cobra venom served as positive control.

TLC/FID assay

Duplicate samples of isolated complement proteins (0.5 μM final concentration) were incubated in a water bath with constant agitation for 2 hr at 37°C in a total vol of 1 ml with 4 m*M* egg phosphatidylcholine, 16 m*M* Triton X-100 (Rohm & Haas), 8 m*M* $CaCl_2$ and 60 m*M* Tris–HCl, pH 7.4. The reaction was stopped by addition of 0.4 ml $CHCl_3$/CH_3OH/acetic acid (2/4/1, v/v/v) and vortexing. Then, 0.4 ml of $CHCl_3$ was added. After vortexing, phase separation was achieved by centrifugation. The aqueous phase was discarded and the organic phase was evaporated to dryness in a vacuum oven at 40°C. The lipids were dissolved in 20 μl $CHCl_3$ and 0.5 μl aliquots were applied to chromatorods. After development in $CHCl_3$/CH_3OH/NH_4OH (70/20/2, v/v/v), the rods were subjected to flame ionization detection in an Iatroscan TH-10 (Iatron) connected with a computing integrator (Spectra Physics).

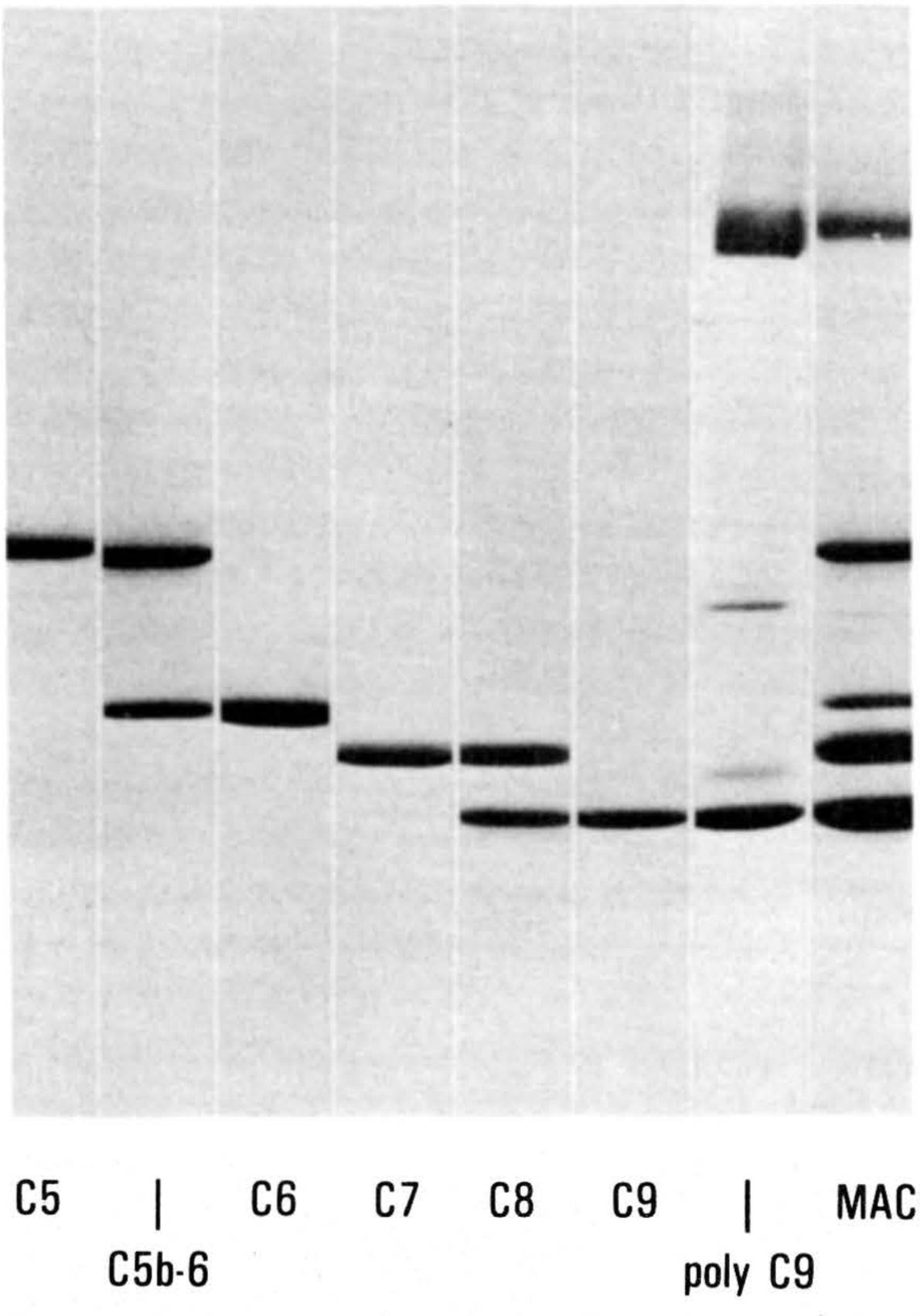

Fig. 1. 2.5–10% gradient SDS-polyacrylamide gel (Laemmli, 1970) stained with Coomassie Blue after electrophoresis under nonreducing conditions of purified human complement proteins C5, C5b–6, C6, C7, C8, C9, poly C9 and MAC (from left to right). The poly C9 shows the SDS-resistant, high molecular weight poly C9 which represents the circularly polymerized C9 (Podack & Tschopp, 1982*b*), the disulfide-linked C9 dimer (Ware & Kolb, 1981), and monomeric C9 which is derived from non-SDS-resistant linearly polymerized C9 (Podack & Tschopp, 1982*b*). Poly C9 and C9 dimer are also seen in the MAC.

Hemolytic assay

This assay for phospholipase A_2 was performed with duplicate samples of isolated complement proteins (approximately 5 μM final concentration) exactly as described recently elsewhere (Vogel *et al.*, 1981). For the analysis of C5 and C5b–6, the KSCN- and hydrazine-treated serum present in the reaction mixture as a source of lipoprotein was replaced by a mixture of isolated lipoproteins as described in the same paper.

Radioactive assay

Duplicate samples of isolated complement proteins (0.25 μM final concentration) were incubated in a water bath with constant agitation for 2 hr at 37°C in a total volume of 1 ml with 0.1 mM egg phosphatidylcholine containing 60 nCi of di[1-^{14}C]palmitoyl-L-α-phosphatidylcholine (sp. act. 114 mCi/mmol) (Amersham), 0.4 mM Triton X-100, 1 mM $CaCl_2$ and 50 mM Tris–HCl, pH 7.4. A parallel experiment was performed in the absence of Triton X-100 with the phospholipid substrate present as sonicated vesicles. The reaction was stopped, the reaction mixture extracted, the aqueous phase discarded, and the organic phase evaporated as described for the TLC/FID assay. The lipids were dissolved in 30 μl $CHCl_3$ and applied to silica gel 60 plates (Merck). After development in $CHCl_3/CH_3OH/H_2O$ (65/25/4, v/v/v) lipids were visualized with iodine vapor and areas corresponding to phosphatidylcholine, lysophosphatidylcholine and fatty acid were scraped off the plate, transferred into scintillation vials containing 10 ml of toluene/Triton X-100/Liquifluor (New England Nuclear) (40/20/3, v/v/v), vigorously vortexed and analyzed for radioactivity.

RESULTS

The highly purified MAC precursor proteins (C5, C5b–6, C6, C7, C8, C9), poly C9, and a mixture of the precursor proteins (molar ratios C5b–6:C7:C8:C9, 1:1:1:6) (Fig. 1) were subjected to the TLC/FID assay and the radioactive assay. In the mixture, MAC assembly commences instantaneously (Podack *et al.*, 1980). No covalent breakdown of phosphatidylcholine could be detected by either assay in the reaction mixtures containing the precursor proteins, poly C9 or the forming MAC (Table 1). Based on an estimated detection limit of 5% hydrolysis (TLC/FID assay) or 1% hydrolysis (radioactive assay) it was calculated that the specific phospholipase A_1 or A_2 activity of each of the tested proteins could not be more than shown in Table 1. Phospholipase C and D activity can also be excluded.*†

* Diglycerides, the reaction product of phospholipase C, exhibit in the neutral TLC solvent a similar R_f value to fatty acids, one of the reaction products of phospholipase A_1 or A_2. Therefore, phospholipase C would have also been detected in the radioactive assay.

† Phospholipase D would have been detected in the TLC/FID assay with the basic TLC solvent by the disappearance of phosphatidylcholine, since the reaction product, phosphatidic acid, remains at the origin. Since no decrease of phosphatidylcholine was observed, phospholipase D activity can also be excluded. In the neutral TLC solvent used with the radioactive assay, phosphatidylcholine and phosphatidic acid migrate closely together. Phospholipase D activity would have escaped detection in this assay.

Table 1. Lack of phospholipase activity of terminal complement proteins

Protein	TLC/FID Assay: Amount/Assay (μg)	TLC/FID Assay: Activity Observed (% Hydrolysis of Phospholipid, 2 hr, 37°C)	TLC/FID Assay: Sensitivity of Assay[a] (μmol/min/mg)	Radioactive Assay: Amount/Assay (μg)	Radioactive Assay: Activity Observed (% Hydrolysis of Phospholipid, 2 hr, 37°C)	Radioactive Assay: Sensitivity of Assay[b] (μmol/min/mg)	Hemolytic Assay: Amount/Assay (μg)	Hemolytic Assay: Activity Observed (% Hemolysis)
C5	100	0	1.7×10^{-2}	50	0	1.7×10^{-4}	34	0
C5b-6	150	0	1.1×10^{-2}	75	0	1.1×10^{-4}	33.5	0
C6	60	0	2.8×10^{-2}	30	0	2.8×10^{-4}	32	0
C7	60	0	2.8×10^{-2}	37.5	0	2.2×10^{-4}	77.5	0
C8	75	0	2.2×10^{-2}	37.5	0	2.2×10^{-4}	47	0
C9	70	0	2.4×10^{-2}	35	0	2.4×10^{-4}	70	0
Poly C9	N.D.	N.D.	N.D.	35	0	2.4×10^{-4}	70	0
MAC	280	0	0.6×10^{-2}	140	0	0.6×10^{-4}	N.A.	N.A.
Phospholipase A_2	0.4	85[c]	- - -	0.04	88[c]	- - -	0.1	75

[a] Maximum specific activity that would have escaped detection based on the limit of detectability of 5% phospholipid hydrolysis.

[b] Maximum specific activity that would have escaped detection based on the limit of detectability of 1% phospholipid hydrolysis.

[c] 100% phospholipid hydrolysis by venom phospholipase A_2 was not reached due to the known product inhibition of this enzyme (Verheij *et al.*, 1981). The enzyme exhibits under intitial rate conditions a specific activity of 600–1000 μmol/min/mg (Deems & Dennis, 1980).

N.D. = Not determined.

N.A. = Not applicable.

The MAC precursor proteins and poly C9 were subjected to a sensitive hemolytic assay for phospholipase A_2 which is based on the synergistic hemolysis with cardiotoxin. Testing of the forming MAC by this assay was precluded since it induces hemolysis. No phospholipase A_2 activity was detected in the proteins tested (Table 1). With 10 ng of purified venom phospholipase A_2 being the limit of detection, the hemolytic assay is almost as sensitive as the TLC/FID assay under the conditions employed here.

DISCUSSION

The possible participation of phospholipase activity in complement-mediated membrane damage has been explored for may years. It was initially proposed that lysophospholipids generated from the phospholipids of serum lipoproteins are involved in the hemolytic activity of complement (Bergenhem, 1938; Isliker, 1958; Fischer & Haupt, 1960; Fischer & Haupt, 1961; Haupt *et al.*, 1963; Fischer, 1964*a*; Fischer, 1964*b*), but this hypothesis did not prove to be correct (Becker, 1964; Phillips & Middleton, 1965; Dalmasso & Müller-Eberhard, 1966). The possibility remained that a complement phospholipase could act directly on the phospholipids of the target membrane. While it was shown that no major covalent breakdown of phospholipids occurs, conflicting results have been reported with regard to a residual enzymatic activity associated with MAC action (Humphrey & Dourmashkin, 1965; Inoue & Kinsky, 1970; Hesketh *et al.*, 1972; Kinoshita *et al.*, 1977; Smith & Becker, 1968; Lachmann *et al.*, 1973; Kinsky *et al.*, 1971; Inoue *et al.*, 1974*a*; Inoue *et al.*, 1974*b*). Very recent results suggest strongly an enzymatic activity in C6 and C7 (Kolb *et al.*, 1982; Kolb & Kolb, 1982). Therefore, we tested the highly purified complement components and the forming MAC for phospholipase activity. No phospholipase activity could be detected employing three different sensitive assays for phospholipase activity: a hemolytic test using natural membranes as phospholipid substrate, the TLC/FID assay with mixed micelles of Triton X-100 and egg phosphatidylcholine at saturating concentration, and the radioactive assay with mixed micelles or phospholipid vesicles containing a low phospholipid amount to achieve a high percentage of hydrolysis with low enzyme activities. While the sensitivity of the radioactive assay could be further increased by increasing the protein concentration and by prolonging the incubation, we believe that any phospholipase activity that might have escaped

detection under the conditions employed would be without biological significance: based on the TLC/FID assay not more than 3.3 molecules of phospholipid could have been cleaved without detection per min at 37°C per molecule of complement protein or 12 molecules of phospholipid per MAC. Based on the more sensitive radioactive assay not more than 0.033 molecules of phospholipid could have been cleaved and escaped detection per minute at 37°C per molecule of complement protein or 0.12 molecules of phospholipid per MAC. Therefore, our results appear to demonstrate that complement-mediated membrane damage does not involve any cleavage of covalent bonds in the membrane phospholipids due to phospholipase A_1, A_2, C or D activity. This is consistent with the current concept of the membranolytic mechanism of the MAC which assumes that complement-mediated membrane damage is exclusively effected by physical interaction of the MAC subunits with the phospholipid bilayer (Esser *et al.*, 1979; Mayer, 1978; Podack *et al.*, 1982*a*).

Phospholipid degradation products found by other investigators using whole serum (Smith & Becker, 1968; Inoue *et al.*, 1974*b*) or partially purified complement proteins (Lachmann *et al.*, 1973; Delâge *et al.*, 1969; Simard *et al.*, 1969; Delâge *et al.*, 1973; Scherer *et al.*, 1976) may have been due to contaminating serum enzymes. The hemolysis of EACl-7 cells by C8 and cardiotoxin (Okada & Campbell, 1974) can be explained by an increased susceptibility of the labile EACl-8 cells to the lytic action of cardiotoxin.

Our results demonstrate also that the sites of C6 and C7, which are inactivated by serine esterase inhibitors (Kolb *et al.*, 1982; Kolb & Kolb, 1982) are not acting on phospholipids. Also, the phenyl laurate esterase activity (Delâge *et al.*, 1969; Simard *et al.*, 1969) and tributyrinase activity (Delâge *et al.*, 1973) ascribed to C7 and possibly to C6 (Delâge *et al.*, 1969) do not represent phospholipase activity. However, these activities may not be due to contaminating enzymes. Phenyl laurate and tributyrin may represent artificial substrates that are cleaved by the putative serine esterase sites. The physiological substrates for C6 and C7 remain to be characterized.

Acknowledgements—The experienced technical assistance of Mrs Kerry Pangburn and Mrs Sarah Patthi in isolating the complement proteins is gratefully acknowledged.

REFERENCES

Ackman R. G. (1981) Flame ionization detection applied to thin-layer chromatography on coated quartz rods. *Meth. Enzym.* **72,** 205–252.

Becker E. L. (1964) Discussion of paper by Fischer, 1964*b*. *Ann. NY Acad. Sci.* **116,** 1069.

Bergenhem B. (1938) Experimentelle Untersuchungen über die spontanen Veränderungen des Blutes *in vitro*. *Acta path. microbiol. scand., Suppl.* **39,** 1–251.

Biesecker G. & Müller-Eberhard H. J. (1980) The ninth component of human complement: Purification and physicochemical characterization. *J. Immun.* **124,** 1291–1296.

Brockerhoff H. (1973) A model of pancreatic lipase and the orientation of enzymes at interfaces. *Chem. Phys. Lipids* **10,** 215–222.

Brockerhoff H. (1974) Lipolytic enzymes. In *Food Related Enzymes. Adv. in Chem. Ser.* **136,** 131–145.

Dalmasso A. P. & Müller-Eberhard H. J. (1966) Hemolytic activity of lipoprotein-depleted serum and the effect of certain anions on complement. *J. Immun.* **97,** 680–685.

Darke P. L., Jarvis A. A., Deems R. A. & Dennis E. A. (1980) Further characterization and *N*-terminal sequence of cobra venom phospholipase A_2. *Biochim. biophys. Acta* **626,** 154–161.

Deems R. A. & Dennis E. A. (1980) Phospholipase A_2 from cobra venom (*Naja naja naja*) EC 3.1.1.4 phosphatide 2-acylhydrolase. *Meth. Enzym.* **71,** 703–710.

deHaas G. F., Sarda L. & Rogers J. (1965) Positional specific hydrolysis of phospholipids by pancreatic lipase. *Biochim. biophys. Acta* **106,** 638–640.

Delâge J.-M., Lehner-Netsch G. & Simard J. (1973) The tributyrinase activity of C7. *Immunology* **24,** 671–678.

Delâge J.-M., Simard J. & Lehner-Netsch G. (1969) Lipase et complément. *Rev. Can. Biol.* **28,** 157–164.

Dijkstra B. W., Dreuth J. & Kalk K. H. (1981) Active site and catalytic mechanism of phospholipase A_2. *Nature, Lond.* **289,** 604–606.

Esser A. F., Kolb W. P., Podack E. R. & Müller-Eberhard H. J. (1979) Molecular reorganization of lipid bilayers by complement: a possible mechanism for membranolysis. *Proc. natn. Acad. Sci., U.S.A.* **76,** 1410–1414.

Fischer H. (1964*a*) Über den Lysemechanismus von Serum-Komplement. *Z. Immunität. Allergieforsch.* **126,** 131–136.

Fischer H. (1964*b*) Lysolecithin and the action of complement. *Ann. N.Y. Acad. Sci.* **116,** 1063–1069.

Fischer H. & Haupt I. (1960) Über das cytolysierende Prinzip von Serum-Komplement. *Naturwissenschaften* **47,** 137.

Fischer H. & Haupt I. (1961) Das cytolysierende Prinzip von Serum-Komplement. *Z. Naturforschung.* **16b,** 321–328.

Fischer W., Heinz E. & Zeus M. (1973) The suitability of lipase from *Rhizopus arrhizus delemar* for analysis of fatty acid distribution in dihexosyl diglycerides, phospholipids and plant sulfolipids. *Hoppe Seyler's Z. physiol. Chem.* **354,** 1115–1123.

Grossman S., Oestreicher G. & Singer T. P. (1974) Determination of the activity of phospholipases A, C and D. *Meth. biochem. Analysis* **22,** 177–204.

Hammer C. H., Wirtz G. H., Renfer L., Gresham H. D. & Tack B. F. (1981) Large scale isolation of functionally active components of the human complement system. *J. biol. Chem.* **256,** 3995–4006.

Haupt I., Fischer H., Rother U. & Rother K. (1963) Absence of lysolecithin formation in C3-deficient rabbit serum. *Nature, Lond.* **200,** 686–687.

Hesketh T. R., Payne S. N. & Humphrey J. H. (1972) Complement and phospholipase C lysis of lipid membranes. *Immunology* **23,** 705–711.

Humphrey J. H. & Dourmashkin R. R. (1965) The lesions in cell membranes caused by complement. *Adv. Immun.* **11,** 75–115.

Inoue K. & Kinsky S. C. (1970) Fate of phospholipids in liposomal model membranes damaged by antibody and complement. *Biochemistry* **9,** 4767–4776.

Inoue K., Takamizawa A., Yano K.-I. & Amano T. (1974*a*) Chemical studies on damages of *Escherichia coli* by the immune bactericidal reaction. I. Release and degradation of phospholipids from damaged bacteria. *Biken's J.* **17,** 127–134.

Inoue K., Yano K.-I. & Amano T. (1974*b*) Chemical studies on damages of *Escherichia coli* by the immune bactericidal reaction. II. Release of phosphatidylethanolamine from a phospholipase A-deficient mutant of *E. coli* during the immune bactericidal reaction. *Biken's J.* **17,** 135–140.

Isliker H. (1958) Unspezifische, humorale Abwehrmechanismen. *Schweiz. med. Wschr.* **88,** 127–132.

Kamer G. & Argos P. (1981) Comparison of the active center geometries in phospholipase, typsin and thermolysin. *Biochim. biophys. Acta* **669,** 93–97.

Kensil C. R. & Dennis E. A. (1979) Action of cobra venom phospholipase A_2 on the gel and liquid crystalline states of dimyristoyl and dipalmitoyl phosphatidylcholine vesicles. *J. biol. Chem.* **254,** 5843–5848.

Kinoshita T., Inoue K., Okada M. & Akiyama Y. (1977) Release of phospholipids from liposomal model membrane damaged by antibody and complement. *J. Immun.* **119,** 73–78.

Kinsky S. C., Bonsen P. P. M., Kinsky C. B., van Deenen L. L. M. & Rosenthal A. F. (1971) Preparation of immunologically responsive liposomes with phosphonyl and phosphinyl analogs of lecithin. *Biochim. biophys. Acta* **233,** 815–819.

Kolb L. M. & Kolb W. P. (1982) Expression of anti-thrombin III (AT III) antigenic determinants by the fluid phase SC5b-9 attack complex of human complement. *Fed. Proc.* **41,** 486.

Kolb W. P., Kolb L. M. & Savary J. R. (1982) Biochemical characterization of the sixth component (C6) of human complement. *Biochemistry* **21,** 294–301.

Kolb W. P. & Müller-Eberhard H. J. (1976) The membrane attack mechanism of complement: the three polypeptide chain structure of the eighth component (C8). *J. exp. Med.* **143,** 1131–1139.

Lachmann P. J., Bowyer D. E., Nichol P., Dawson R. M. C. & Munn E. A. (1973) Studies on the terminal stages of complement lysis. *J. Immun.* **24,** 135–145.

Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, Lond.* **227,** 680–685.

Mayer M. M. (1972) Mechanism of cytolysis by complement. *Proc. natn. Acad. Sci. U.S.A.* **69,** 2954–2958.

Mayer M. M. (1978) Complement, past and present. *Harvey Lecture* **72,** 139–193.

Okada H. & Campbell W. (1974) Immune hemolysis and a basic peptide from cobra venom. *J. Immun.* **113,** 1647–1649.

Phillips G. B. & Middleton E. (1965) Studies on lysolecithin formation in immune hemolysis. *J. Immun.* **94,** 40–46.

Podack E. R., Esser A. F., Biesecker G. & Müller-Eberhard H. J. (1980) Membrane attack complex of complement. A structural analysis of its assembly. *J. exp. Med.* **151,** 301–313.

Podack E. R., Kolb W. P., Esser A. F. & Müller-Eberhard H. J. (1979) Structural similarities between C6 and C7 of human complement. *J. Immun.* **123,** 1071–1077.

Podack E. R. & Müller-Eberhard H. J. (1980) Limited proteolysis of C5b–6: functional stability of the degraded complex. *J. Immun.* **124,** 332–336.

Podack E. R., Müller-Eberhard H. J., Horst H. & Hoppe W. (1982*a*) Membrane attack complex of complement (MAC): three-dimensional analysis of MAC-phospholipid vesicle recombinants. *J. Immun.* **128,** 2363–2357.

Podack E. R. & Tschopp J. (1982*a*) Polymerization of the ninth component of complement (C9): formation of poly C9 with a tubular ultrastructure resembling the membrane attack complex of complement. *Proc. natn. Acad. Sci. U.S.A.* **79,** 574–578.

Podack E. R. & Tschopp J. (1982*b*) Characterization of poly C9 and analysis of its pore forming membrane binding site. *Fed. Proc. Fed. Am. Socs exp. Biol.* **41,** 486.

Podack E. R., Tschopp J. & Müller-Eberhard H. J. (1982*b*) Molecular organization of C9 within the membrane attack complex of complement: induction of circular C9 polymerization by the C5b–8 assembly. *J. exp. Med.* **156,** 268–282.

Scherer R., Huber-Friedberg W., Salem A. & Ruhenstroth-Bauer G. (1976) Phospholipase A_2 activity in human γ-globulin fraction. *Hoppe Seyler's Z. physiol. Chem.* **357,** 897–902.

Simard J., Lehner-Netsch G. & Delâge J.-M. (1969) Activité lipasique de C7. *Rev. Can. Biol.* **28,** 229–231.

Singleton W. S., Gray M. S., Brown M. L. & White J. L. (1965) Chromatographically homogeneous lecithin from egg phospholipids. *J. Am. Oil Chem. Soc.* **42,** 53–56.

Slotboom A. J., deHaas G. H., Bonsen P. P. M., Burbach-Westerhuis G. J. & van Deenen L. L. M. (1970) Hydrolysis of phosphoglycerides by purified lipase preparations. I. Substrate-, positional- and stereospecificity. *Chem. Phys. Lipids* **4,** 15–29.

Smith J. K. & Becker E. L. (1968) Serum complement and the enzymatic degradation of erythrocyte phospholipid. *J. Immun.* **100,** 459–474.

Tschopp J., Müller-Eberhard H. J. & Podack E. R. (1982) Formation of transmembrane tubules by spontaneous polymerization of the hydrophilic complement protein C9. *Nature, Lond.* **298,** 534–538.

Verheij H. J., Slotboom A. J. & deHaas G. H. (1981) Structure and function of phospholipase A_2. *Rev. Physiol. Biochem. Pharmacol.* **91,** 91–203.

Vogel C.-W., Plückthun A., Müller-Eberhard H. J. & Dennis E. A. (1981) Hemolytic assay for venom phospholipase A_2. *Analyt. Biochem.* **118,** 262–268.

Ware C. F. & Kolb W. P. (1981) Assembly of the functional membrane attack complex of human complement: formation of disulfide-linked C9 dimers. *Proc. natn. Acad. Sci. U.S.A.* **78,** 6426–6430.