HUMAN COMPLEMENT IN THE ARACHIDONIC ACID TRANSFORMATION PATHWAY IN PLATELETS*

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We have found that complement is activated during blood coagulation (1). This activation was characterized by the development of ultrastructural lesions on the platelet surface subsequent to physiologic clotting. The appearance of the lesions was dependent on the presence of thrombin and of C3. In further studies, the role of complement in thrombin-mediated platelet function was investigated (2, 3). It was found that thrombin-mediated platelet aggregation and release of serotonin was significantly enhanced in the presence of complement. Only complement components C3, C5, C6, C7, C8, and C9 were required for this reactivity. No known activating mechanism of either the classical pathway or alternative pathway was required. Thus, a new pathway of activation of complement was described—one which required thrombin, the platelet surface, and entered the known complement sequence at the C3 stage. Activation of this pathway led to the production of C5b-9 complexes that were visualized as typical lesion formation on the platelet surface (3). During these studies it was shown that the complement effect was inhibited by aspirin and indomethacin, drugs known to inhibit platelet cyclooxygenase. Thus the possibility existed that the complement effect might be mediated via the arachidonic acid transformation pathway with production of thromboxane A_2.

The purpose of this study was to elucidate the role of complement in the arachidonic acid transformation pathway in platelets.

Materials and Methods

Preparation of Washed Platelets and Platelet Membranes. A suspension of human platelets was obtained using the Ardlie buffer system modified as previously described (2). Platelet membranes were prepared according to a published method (4).

Preparation of Complement Components. C3 (5), C4 (6), C5 (7), C6, C7 (8), C8 (9), and C9 (10) were prepared by methods described earlier. In addition, in some experiments, purified complement components prepared by Cordis Laboratories Inc., Miami, Fla. were utilized. Before use, the complement components were dialyzed overnight against cacodylate buffer, pH 7.4 (2).

Radiolabeling of the Third (C3) and Fifth (C5) Components of Complement. C3 and C5 were labeled with ^{125}I by the method of McConahey and Dixon (11). The specific activity of each component was ~100,000 cpm/μg.

Platelet Aggregation. Assays for platelet aggregation were performed in a Payton dual channel aggregometer equipped with a Riken Denshi recorder (Payton Associates, Inc., Buffalo, N. Y.). Washed platelets were suspended at 200,000/μl in Ardlie II buffer in which bicarbonate was

* Supported by grant HL 18828 from the Specialized Center for Thrombosis Research, National Institutes of Health and by the Arnold R. Krakower Foundation.

‡ Recipient of a Faculty Research Award from the American Cancer Society.

replaced with Tris (2). Sodium arachidonate (Nuchek Prep., Inc., Elysian, Minn.) at various concentrations in 10-μl vol was added to 0.3 ml platelets. The sodium arachidonate used in these experiments was prepared under nitrogen. The platelet mixtures were stirred for 5 min while aggregation responses were recorded, and samples then were taken for release measurements. The sodium salt of both oleic and linoleic acid (Sigma Chemical Co., St. Louis, Mo.) were also utilized.

Release of [^{14}C]Serotonin. Platelets suspended in plasma were labeled with [^{14}C]serotonin by the method of Valdorf-Hansen and Zucker (12). The radiolabeled platelets were then washed in the usual way (2, 3). For the experiment, aliquots of various reagents were added to 0.3 ml of the washed platelet suspension. Aggregation was recorded for 5 min, and then the tube was rapidly centrifuged and the supernate removed. ^{14}C was counted in both the supernate and the cell button in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) and the percentate of release was calculated.

Release of Lactic Dehydrogenase (LDH).[1] LDH release was determined by the method of Wroblewski and La Due (13). 1 U was expressed as decrease in OD at 340 μm of 0.001/min per ml. A control sample giving 100% lysis was obtained by the addition of 0.1% Triton X-100.

Assay for Thromboxane B_2 (TXB_2). The amounts of thromboxane A_2 (TXA_2) released by the platelet suspensions during stimulation with aggregating agents in the presence and absence of complement components were measured by radioimmunoassay for TXB_2, the stable metabolite of TXA_2. Conversion to this metabolite was accomplished by permitting the platelet supernates to remain in aqueous solution. The radioimmunoassay for TXB_2 was developed using antibodies produced in rabbits to a carbodiimide conjugate of synthetic TXB_2 with keyhole limpet hemocyanin. Details of this assay will be presented elsewhere. (R. Levin, B. B. Weksler, K. Tack-Goldman, and E. A. Jaffe. Manuscript in preparation.) Briefly, 100 μl of platelet supernate, 100 μl of anti-TXB_2 antiserum (1:4,800) and 100 μl of [^{3}H]TXB_2 (150 Ci/mmol sp act; 10,000 dpm; New England Nuclear, Boston, Mass.) were incubated together at room temperature for 18 h. Separation was accomplished by addition of 100 μl protein A-containing staphylococci (Pansorbin; 1:10 dilution; Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) and liquid scintillation counting of an aliquot of supernate after centrifugation of the precipitated antigen-antibody complexes. Assay data were analyzed by the four parametric method of Rodbard. The range of sensitivity for TXB_2 was 3–1,000 pg, with a midpoint of 30–40 pg. Duplicate samples were assayed at two or three dilutions.

Uptake of ^{125}I-C3 and ^{125}I-C5 by Platelet Membranes. Platelet membranes prepared as described previously (4) were divided into four 1-ml aliquots. To the first aliquot was added 50 CH_{50} U (CH_{50} U, the amount of any complement required to produce 50% lysis of antibody-sensitized sheep cells) each of C5, C6, C7, C8, and C9 and ^{125}I-C3 (60 μg containing 4.2×10^6 cpm). To the second aliquot was added 50 CH_{50} U of each C3, C6, C7, C8, and C9 and ^{125}I-C5 (10 μg containing 2×10^6 cpm). Sodium arachidonate was added to both aliquots at a final concentration of 100 μM. Aliquots 3 and 4 contained the same complement components as did 1 and 2, but did not contain sodium arachidonate. The mixtures were incubated at 37°C for 1 h, and then after an extensive washing procedure, the radioactivity in the membrane pellet was determined. The platelet membranes were dissolved in 1 N NaOH and the protein concentration was determined.

Preparation of $F(ab')_2$ Fragments of Antisera. The γ-globulin fractions were prepared from monospecific antiserum to C3, C4, C5, C6, and C9, and human serum albumin prepared in rabbits and goat anti-rabbit γ-globulin by ammonium sulfate precipitation followed by triethylaminoethyl-cellulose column chromatography with phosphate buffer, pH 8.0, 0.0175 M. The purified γ-globulin preparations were digested with pepsin at various concentrations from 0.1 to 1% by the method of Nisonoff et al. (14). Antibody activity of the digested protein was tested by Ouchterlony analysis and degree of digestion was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It was found with each antibody that digestion with 0.4% pepsin yielded a totally digested protein which retained full antibody activity.

Ferritin Conjugation of Goat Anti-Rabbit γ-Globulin Serum. The $F(ab')_2$ fragment of the goat anti-

[1] *Abbreviations used in this paper:* CH_{50} U, the amount of any complement component required to produce 50% lysis of antibody-sensitized sheep cells. DOC, deoxycholate; LDH, lactic dehydrogenase; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2.

rabbit γ-globulin serum was conjugated with ferritin by the method of Tawde and Ram (15). Free ferritin and unconjugated γ-globulin were separated from conjugated γ-globulin by Pevikon (Mercer Consolidated Corp., Yonkers, N. Y.) block electrophoresis (16).

Localization of Arachidonate-mediated Platelet-bound C3 and C5 by Electron Microscopy. 200 CH_{50} U each of C3, C5, C6, C7, C8, and C9 and sodium arachidonate at a final concentration of 100 μM were added to 15 ml washed platelet suspension at 200,000/μl. The mixture was placed on a rotator for 20 min at 37°C. The platelets were washed three times with saline and then divided into three equal aliquots of 1 ml. To the first aliquot was added 0.3 ml of the $F(ab')_2$ fraction of anti-C3; to the second aliquot, the similar fraction of anti-C5; and to the third aliquot saline was added. The mixtures were incubated at 37°C for 1 h with occasional mixing. After three washes with saline, 0.2 ml of ferritin-conjugated$F(ab')_2$ fragment of goat anti-rabbit γ-globulin was added to the resuspended platelets. These mixtures were incubated at 37°C for 1 h with occasional mixing. The platelets were washed three times in saline and after the last wash were resuspended in 0.5 ml water. The platelets were then lysed by freezing and thawing three times. The resultant platelet pellet was washed twice with saline diluted 1:10 and buffered to pH 6.5, then applied unstained to a copper grid coated with collodion and carbon and viewed under a Philips 301 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.) at 80 kv.

Preparation of Arachidonate-mediated Platelet Membrane-bound C5b-9 Complexes. Platelet membranes from 9 U of blood were utilized (4). 1 ml modified Ardlie II buffer (2) was added to the membrane button. The membranes were thoroughly mixed and 0.25 ml was aliquoted into each of two tubes. 4.75 ml of the same buffer was added to both tubes. To the first tube was added 90 μg C5 (4.0×10^7 cpm) and 400 CH_{50} units of each C6, C7, C8, and C9, followed by sodium arachidonate at a final concentration of 100 μM. The second aliquot contained the same amount of complement components but no arachidonate. Both tubes were clipped onto a rotator and incubated for 30 min at 37°C. The same method was utilized to elute the putative C5b–9 complexes as was previously described (3). Briefly, the complexes were eluted with 10% deoxycholate (DOC) (Sigma Chemical Co.). The eluate was applied to a Bio-Gel A15m column (Bio-Rad Laboratories, Richmond, Calif.). The first peak of protein to elute from this column was subjected to sucrose density gradient ultracentrifugation. The material obtained from the gradient with a sedimentation coefficient of 33S was processed for electron microscopy (3) and viewed in a Philips 301 electron microscope.

Results

Effect of Complement on Sodium Arachidonate-mediated Release of [^{14}C]Serotonin. Table I shows the results of 10 experiments in which sodium arachidonate-mediated release of ^{14}C-serotonin from washed platelets was measured in the presence and absence of complement. In every experiment, addition of complement components C5, C6, C7, C8, and C9 led to an increase in the percent release of serotonin. 4 CH_{50} U of each component were found to be optimal in augmenting serotonin release. The concentrations of sodium arachidonate employed were 30–70 μM and in this range, <10% platelet lysis resulted. The degree of enhancement of serotonin release varied from 46 to 554% ($P < 0.001$). The factors influencing the variability of the response in different platelet preparations was not determined. All five components, C5, C6, C7, C8, and C9, were required for an optimal enhancement effect. Experiments were performed using C5 alone, C5, C6, and C7 alone, and C8 and C9 alone. Under these conditions, suboptimal enhancement of serotonin release was obtained. This pattern was similar to that observed in our previous studies with thrombin (2). In no case did addition of C5–C9 lead to an increase in platelet lysis. Pretreatment of platelets with aspirin (100 μM) totally inhibited the arachidonate-mediated serotonin release both in the absence and presence of complement. Addition of C5–C9 induced no enhancement of ^{14}C

TABLE I
Arachidonic Acid-mediated Release of [^{14}C]Serotonin in the Presence and Absence of Complement

Experiment	Percent release of serotonin		Percent enhancement
	Arachidonic acid* alone	Arachidonic acid plus C5–9‡	
1	31	52	67.7
2	22	69	213.6
3	35	79	125.7
4	11	32	190.0
5	12	35	191.6
6	32	47	46.8
7	44	88	100.0
8	16	28	75.0
9	11	71	554.5
10	26	44	69

* Concentration of arachidonic acid varied from 30 to 70 μM.
‡ 4 CH_{50} U each of C5, C6, C7, C8, and C9.

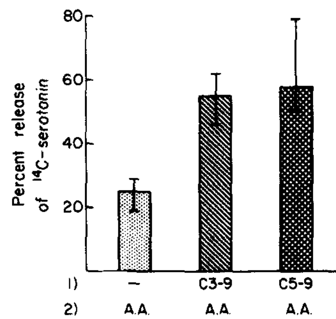

FIG. 1. Effect of purified complement components on arachidonate-mediated release of [^{14}C]-serotonin. A.A.: sodium arachidonate (50 μM); C3–9: complement components C3, C5, C6, C7, C8, and C9; C5–9: complement components C5, C6, C7, C8 and C9; 1 and 2 indicate sequence of addition.

serotonin release mediated by either oleic or linoleic acid (in the dose range of 70–120 μM).

Because complement components C3–C9 are required for enhancement of thrombin-mediated platelet release of serotonin, experiments were performed to determine whether addition of C3 might increase the release obtained with C5–C9. As indicated in Fig. 1, no increment in serotonin release was obtained when C3 was added to the C5-9 mixture. Because it has been shown (2) that complement proteins are present on the platelet surface, the possibility arose whether membrane-bound C3 might be participating in the complement-mediated reaction in the absence of exogenously added C3. To answer this question, platelets were incubated with the $F(ab')_2$ fraction of anti-C3 before exposure to sodium arachidonate and C5–C9. As shown in Fig. 2, incubation of platelets with anti-C3 did not inhibit serotonin release mediated by C5–C9.

As was the case with the complement-dependent enhancement of thrombin-mediated platelet function, no known activating mechanism of the classical or alternative

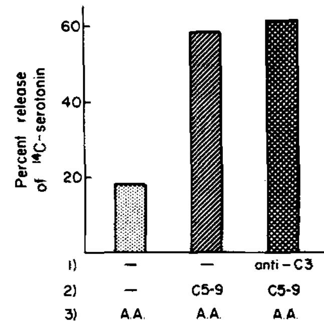

FIG. 2. Effect of anti-C3 on arachidonate-mediated release of [^{14}C]serotonin in the presence of C5–9. Anti-C3: F(ab')$_2$ fragments of anti-C3. A.A.: sodium arachidonate (50 μM); C5–9: complement components C5, C6, C7, C8, and C9; 1, 2, and 3 indicate sequence of addition.

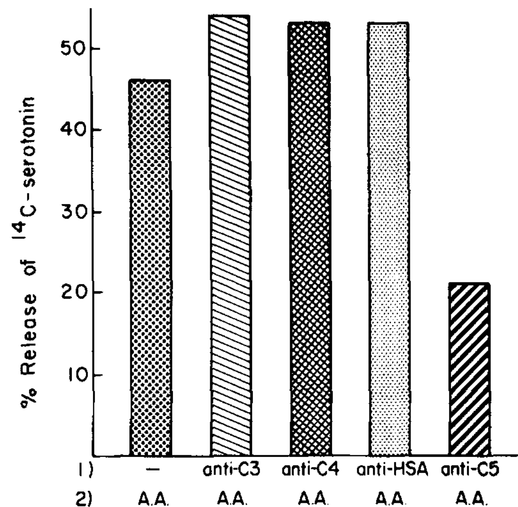

FIG. 3. Effect of various antibodies on arachidonate-mediated release of [^{14}C]serotonin in the absence of exogenously added complement. A.A.: sodium arachidonate (70 μM); anti-HSA: anti-human serum albumin. In each case the F(ab')$_2$ fragment of the antibody was utilized. 1 and 2 indicate sequence of addition.

pathway was required for the complement-dependent enhancement of arachidonate-mediated platelet release.

Role of Platelet Membrane-bound Complement in the Absence of Exogenously Added Complement Proteins. Because complement components are present on the platelet surface (2), the possibility arose as to whether there is enough surface-bound complement to mediate the enhancement effect in the absence of exogenously added protein. To elucidate this point, washed platelets were incubated with various antibodies before exposure to sodium arachidonate. No complement components were added. As shown in Fig. 3, the F(ab')$_2$ fractions of anti-C3, anti-C4, and anti-human serum albumin caused no inhibition of release of serotonin. However, inhibition was obtained if the platelets were first incubated with anti-C5 before exposure to arachidonate. Inhibition was also obtained if the platelets were first incubated with anti-C6 or anti-C9 (data not shown). Thus, there was sufficient complement proteins on the platelet surface to

TABLE II
Arachidonic Acid-mediated Release of TXB_2 in the Presence and Absence of Complement

Experiment	TXB_2		Percent enhancement
	Arachidonic acid* alone	Arachidonic acid plus C5–9‡	
	ng/ml		
1	51.9	90.7	74.7
2	178.0	372.0	108.9
3	52.1	154.0	195.5
4	91.0	181.1	99.9

* 70 μM arachidonic acid.
‡ 4 CH_{50} U of each C5, C6, C7, C8, and C9.

TABLE III
Arachidonate-mediated Uptake of C3 and C5 by Platelets

Uptake mediated by	Number of molecules/μg membrane protein	
	C3	C5
Sodium arachidonate (100 μM)	None	3.06×10^9

support a complement-mediated reaction in the absence of exogenously added complement components.

Effect of Complement on Arachidonate-mediated Release of TXB_2. In every experiment, the addition of C5–C9 led to an increase in arachidonate-mediated release of TXB_2. The enhancement of release ranged from 74 to 195% (Table II).

Sodium Arachidonate-mediated Lysis of Washed Platelets. It was found that high concentrations of sodium arachidonate (>100 μM) led to lysis of washed platelets as determined by release of LDH. This observation confirmed data published by Ts'ao and Holly (17). However, at concentrations of arachidonate of ≤70 μM, <10% release of LDH was obtained. In no case did addition of complement lead to an increase in release of LDH.

Sodium Arachidonate-mediated Uptake of ^{125}I-C3 and ^{125}I-C5 by Platelets. As shown in Table III, incubation of platelet membranes with sodium arachidonate and complement components C3–C9 in which either C3 or C5 was labeled with ^{125}I led to a specific uptake of C5 of 3.06×10^9 molecules/μg of membrane protein. The uptake of C3 was indistinguishable for that of the control sample lacking arachidonate.

Morphological Demonstration of Arachidonate-Mediated Uptake of C5. An indirect ferritin assay was utilized for this study. Platelets, following incubation with sodium arachidonate and components C3–C9, were first incubated with the $F(ab')_2$ fractions of either anti-C3 or anti-C5 and subsequently exposed to ferritin-conjugated goat antibody to rabbit γ-globulin. Fig. 4A demonstrates the uptake and distribution of the ferritin label when anti-C5 was employed. As shown in Fig. 4B, no ferritin label was present when the antibody employed was anti-C3. These data thus confirm morphologically that arachidonate-mediates uptake of C5, but not of C3.

Physiocochemical and Morphological Demonstration of the Presence of C5b–9 Complexes on the

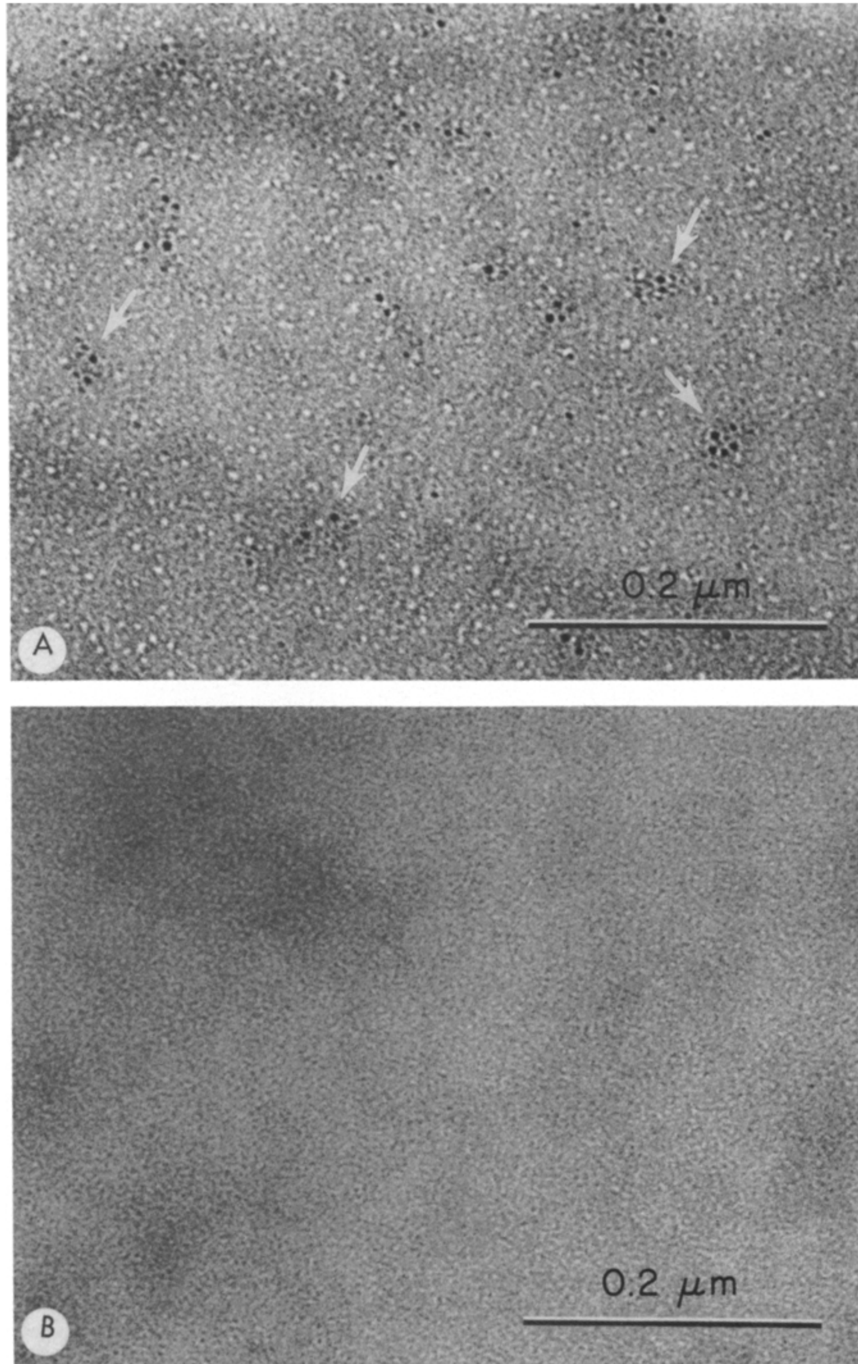

FIG. 4. Preparation of platelet membranes incubated with arachidonate and complement components C3–C9 reacted with the $F(ab')_2$ fragment of either anti-C5 (A) or anti-C3 (B) then subsequently with ferritin-conjugated goat anti-rabbit γ-globulin. Unstained, × 198,000. Arrows in (A) indicate typical clumps of ferritin molecules. No ferritin was seen in membrane preparation that had been reacted with anti-C3 (B).

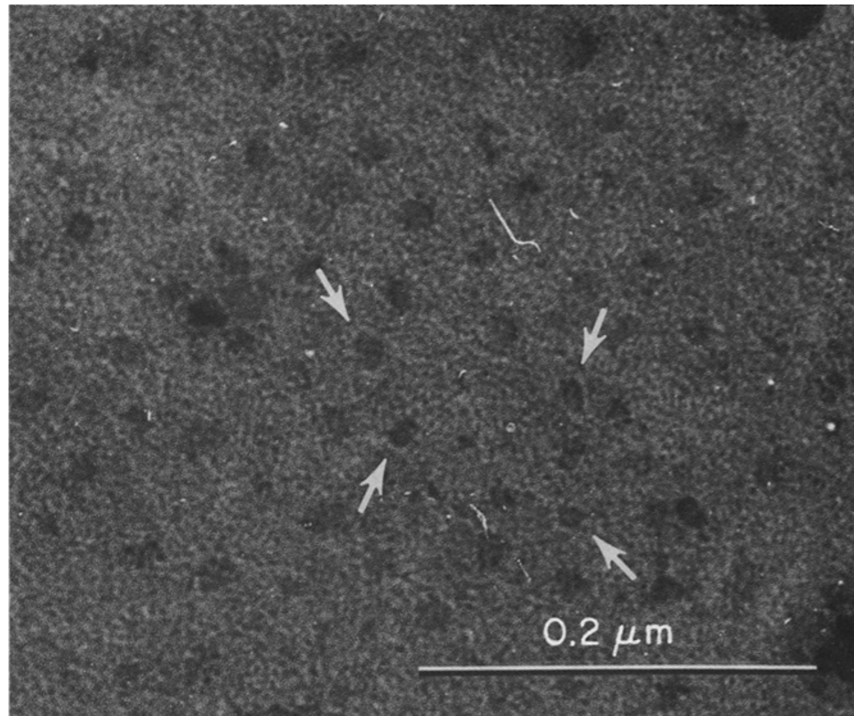

FIG. 5. Electron microscopical analysis of 33S fraction from sucrose density gradient (see Materials and Methods). The sample was dialyzed to remove sucrose, stained with 2% sodium silicotungstate, and then applied directly to a grid coated with collodion and carbon. × 264,000. The micrograph illustrates a suspension of C5b-9 dimers. The arrows indicate C5b-9 dimers which are settled flat on the grid.

Platelet Surface, Their Formation, and Uptake Mediated by Sodium Arachidonate. Platelet membranes were incubated with sodium arachidonate and complement components C5-C9 in which C5 was radiolabeled. The putative C5b-9 complexes were eluted with 10% DOC. When the eluate was applied to a Bio-Gel A15m column, complex formation was indicated by a peak of radiolabeled protein which eluted immediately after the position of a blue dextran marker. Material from this peak was subjected to sucrose density gradient ultracentrifugation, and C5b-9 complex formation was indicated by the presence of a peak of radiolabel (^{125}I-C5) with a sedimentation coefficient of 33S. When the appropriate fraction from the density gradient was visualized under the electron microscope (Fig. 5), it was found to be morphologically identical to the C5b-9 dimer previously reported (18). No complex formation was indicated in the control sample lacking arachidonate.

Discussion

This study was prompted by our earlier observations (2, 3) that thrombin-mediated platelet aggregation and release are significantly increased in the presence of complement and that the complement effect is inhibited by aspirin and indomethacin (3). Because these drugs are known inhibitors of cyclo-oxygenase, this observation led us to postulate that the complement effect might be mediated via the arachidonic acid

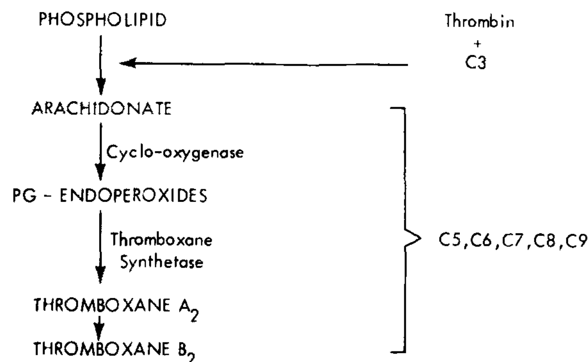

FIG. 6. Scheme showing the potential interrelations of the arachidonic acid transformation pathway and the various components of complement. PG, prostaglandin.

transformation pathway. Thus, in this study, we attempted to elucidate the role of the complement system in this pathway.

It was found that release of serotonin and TXB_2 synthesis mediated by exogenous arachidonate was significantly enhanced in the presence of complement. Only purified complement components C5, C6, C7, C8, and C9 were required for this reactivity. The demonstration of C5b–9 complexes in an eluate from platelet membranes that had interacted with arachidonic acid and complement further documents the participation of these proteins in the arachidonate-stimulated platelet response. No known activating mechanisms of the classical pathway or alternative pathway were required, nor was C3. Further, it was found that platelet-bound complement components play an essential role in modulating arachidonate-mediated sertonin release even in the absence of exogenously added complement.

It was of interest that only complement components C5–9 were required for the enhancement effect of the arachidonic acid pathway. The complement enhancement of thrombin-mediated platelet function requires complement components C3–C9, and this effect is totally inhibited by aspirin (3). Assuming that the metabolism of exogenous arachidonate is similar to that of the endogenous fatty acid released from cell phospholipid, the possibility exists that C3 in association with thrombin is required for mobilization of arachidonic acid from the phospholipid of the platelet membrane. Once the arachidonic acid is mobilized, C3 is no longer required, C5–C9 being sufficient to modulate this pathway leading to enhanced production of TXB_2. A scheme showing the potential interrelations of the arachidonic acid transformation pathway and the various complement components is shown in Fig. 6. A subject for further study is to determine whether C3 alone is required for mobilization of endogenous arachidonic acid or whether C5–C9 are additionally required at this step. It is not known as yet how the assembly of C5b-9 complexes on the platelet surface can directly influence platelet function. The possibility exists that the formation of the membrane attack complex (C5b–9) initiates a transmembrane signal that modulates the arachidonic acid transformation pathway leading to the increased production of TXB_2. It is of some interest that the cyclo-oxygenase and thromboxane synthetase enzyme systems are located in different membrane compartments in the platelet (19). The precise molecular step at which the complement-mediated facilitation occurs remains to be determined.

The functional physiologic significance of these findings in relation to the hemostatic process is best exemplified by the description of two recently reported patients, one deficient in C6, the other in C7 (20). The C6-deficient patient was subject to repeated epistaxis. This patient's platelets in platelet-rich plasma gave reduced aggregation and serotonin release in response to thrombin when compared to normal platelets. Addition of purified C6 corrected the abnormality. The platelets of the C7-deficient patient also exhibited decreased thrombin-mediated aggregation, which was corrected by the addition of purified C7. Complement may play an important role in modulating the fine tuning of the platelet membrane response to physiologically activating stimuli.

To date, the facilitating role of complement on the arachidonic acid transformation pathway has been studied only in platelets. The arachidonic acid transformation pathway plays a major role in influencing cellular behavior in other systems including endothelial cells (21), basophils (22), mast cells (23, 24), polymorphonuclear leukocytes, and macrophages (25, 26) among others. It seems reasonable that complement components may also serve as regulatory modulators of arachidonic acid mobilization and transformation in these different cell systems. These potential relations remain a subject for further study.

Summary

Arachidonate-mediated release of ^{14}C serotonin and thromboxane B_2 (TXB_2) is significantly enhanced in the presence of complement. Only purified complement components C5, C6, C7, C8, and C9 are required for this reactivity. No known activating mechanism of the classical or alternative pathway is required, nor is C3. In the absence of exogenously added complement, platelet membrane-bound complement components play an essential role in modulating arachidonate-mediated serotonin release. Incubation of platelet membranes with arachidonate and C5–C9 led to the production of dimers of the membrane attack complex (C5b–9) on the platelet surface. These macromolecular complexes were eluted from the platelet membrane and were identified physicochemically and morphologically.

The possibility arises that C3 in association with C5–C9 is required for mobilization of the arachidonic acid from the phospholipid of the platelet membrane. Once the arachidonic acid is mobilized, C3 is no longer required, C5–C9 being sufficient to modulate this pathway leading to enhanced production of TXB_2.

The authors thank Ms. Linda Metakis, Mr. Randall Ewald, Mr. Fred Clark, Ms. Karen Tack-Goldman, and Ms. Barbara Ferris for skillful technical assistance. They also thank Ms. Ruth Leviton for her secretarial assistance in the preparation of the manuscript.

Received for publication 11 July 1980 and in revised form 1 October 1980.

References

1. Polley, M. J., and R. L. Nachman. 1975. Ultrastructural lesions on the surface of platelets associated with either blood coagulation or with antibody-mediated immune injury. *J. Exp. Med.* **141**:1261.
2. Polley, M. J., and R. Nachman. 1978. The human complement system in thrombin-mediated platelet function. *J. Exp. Med.* **147**:1713.

3. Polley, M. J., and R. L. Nachman. 1979. Human complement in thrombin-mediated platelet function. Uptake of the C5b-9 complex. *J. Exp. Med.* **150:**633.
4. Nachman, R. L., and E. A. Jaffe. 1975. Subcellular platelet factor VIII antigen and von Willebrand factor. *J. Exp. Med.* **141:**1101.
5. Nilsson, U. R., and H. J. Müller-Eberhard. 1965. Isolation of β_{1F} globulin from human serum and its characterization as the fifth component of complement. *J. Exp. Med.* **122:**277.
6. Müller-Eberhard, H. J., and C. E. Biro. 1963. Isolation and description of the fourth component of human complement. *J. Exp. Med.* **118:**447.
7. Nilsson, U. R., R. H. Tomar, and F. B. Taylor, Jr. 1972. Additional studies on human C5. Development of a modified purification method and characterization of the purified product by polyacrylamide gel electrophoresis. *Immunochemistry.* **9:**709.
8. Podack, E. R., W. P. Kolb, and H. J. Müller-Eberhard. 1976. Purification of the sixth and seventh component of human complement without loss of hemolytic activity. *J. Immunol.* **116:**263.
9. Manni, J. A., and H. J. Müller-Eberhard. 1969. The eighth component of human complement (C8): isolation, characterization and hemolytic efficiency. *J. Exp. Med.* **130:** 1145.
10. Hadding, U., and H. J. Müller-Eberhard. 1969. The ninth component of human complement: isolation, description and mode of action. *Immunology.* **16:**719.
11. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of protein for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* **29:**185.
12. Valdorf-Hanson, J. F., and M. B. Zucker. 1971. Effect of temperature and inhibitors on serotonin-^{14}C release from human platelets. *Am. J. Physiol.* **220:**105.
13. Wroblewski, F., and J. S. LaDue. 1955. Lactic dehydrogenase activity in blood. *Proc. Soc. Exp. Biol. Med.* **90:**210.
14. Nisonoff, A., G. Markus, and F. C. Wissler. 1960. Separation of univalent fragments of rabbit antibody by reduction of disulfide bonds. *Arch. Biochem.* **89:**230.
15. Tawde, S. S., and J. Sri Ram. 1962. Conjugation of antibody to ferritin by means of p,p′-difluro-M,M′-dinitrodiphenyl sulphone. *Arch. Biochem. Biophys.* **97:**429.
16. Müller-Eberhard, H. J. 1960. A new supporting medium for preparative electrophoresis. *Scand. J. Clin. Lab. Invest.* **12:**33.
17. Ts'ao Chung-hsin, and C. M. Holly. 1979. Arachidonic acid causes lysis of human platelets in an artificial medium: protection by plasma. *Prostaglandins.* **17:**775.
18. Biesecker, G., E. R. Podack, C. A. Halverson, and H. J. Müller-Eberhard. 1979. C5b-9 dimer: isolation from complement-lysed cells and ultrastructural identification with complement-dependent membrane lesions. *J. Exp. Med.* **149:**448.
19. White, J. G., and J. M. Gerrard. 1980. The cell biology of platelets. *In* The Cell Biology of Inflammation. G. Weissman, editor. Elsevier North-Holland, Inc., New York. 83.
20. Wautier, J. L., A. P. Peltier, and J. P. Caen. 1979. Complement (C6) and platelet activation by thrombin in humans. *Thromb. Res.* **15:**589.
21. Weksler, B. B., A. J. Marcus, and E. A. Jaffe. 1977. Synthesis of PGI_2 (Prostacyclin) by cultured human and bovine endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:**3922.
22. Marone, G., A. Kagery-Sobotka, and L. M. Lichtenstein. 1979. Effects of arachidonic acid and its metabolites on antigen-induced histamine release from human basophils in vitro. *J. Immunol.* **123:**1669.
23. Roberts, L. J., R. A. Lewis, R. Hansbrough, K. F. Austen, and J. A. Oates. 1978. Biosynthesis of prostaglandins, thromboxanes and 12-hydroxy-5,8,10,14-eicosatetraenoic acid by rat mast cells. *Fed. Proc.* **37:**908.
24. Lewis, R. A., S. T. Holgate, L. J. Roberts, J. F. Maquire, J. A. Oates, and K. F. Austen. 1979. Effects of indomethacin on cyclic nucleotide levels and histamine release from rat serosal mast cells. *J. Immunol.* **123:**1663.

25. Higgs, G. A., S. Bunting, S. Moncada, and J. R. Vane. 1976. Polymorphonuclear leukocytes produce Thromboxane A_2-like activity during phagocytosis. *Prostaglandins.* **12**:749.
26. Morley, J., M. A. Bray, R. W. Jones, D. H. Nugteren, and D. A. van Dorp. 1979. Prostaglandin and Thromboxane production by human and guinea-pig macrophages and leukocytes. *Prostaglandins.* **17**:729.