THROMBOSIS RESEARCH 38; 491-503, 1985
0049-3848/85 $3.00 + .00 Printed in the USA.
Copyright (c) 1985 Pergamon Press Ltd. All rights reserved.

ACTIVATION OF FACTOR B OF THE COMPLEMENT SYSTEM BY KALLIKREIN AND ITS LIGHT CHAIN

P.S. Hiemstra, M.R. Daha and B.N. Bouma*
Department of Nephrology, University Hospital Leiden and Department of Haematology (*), University Hospital Utrecht, The Netherlands.

(Received 17.9.1984; Accepted in revised form 25.2.1985
by Editor G.A. Marguerie)

ABSTRACT

The cleavage of factor B, a protein of the alternative pathway of complement, by kallikrein was studied. Like factor $\bar{D}$, kallikrein can cleave B to generate the alternative pathway C3 convertase C3bBb. When this convertase was formed on erythrocytes previously coated with C3b, lysis was observed indicating that a functionally active C3 convertase was formed. B was also cleaved by kallikrein in the presence of fluid phase C3b, and this resulted in B fragments comparable in size to those generated in the presence of $\bar{D}$. The capacity of kallikrein to cleave B is localised in the light chain of the kallikrein molecule, which is the same chain of kallikrein that is responsible for its other enzymatic activities. Since on a molar basis $\bar{D}$ is much more active then kallikrein in cleaving B, a physiological role for B activation by kallikrein is only likely under certain conditions, and still has to be established.

INTRODUCTION

Plasma kallikrein is a serine protease that participates in haemostatic and inflammatory reactions. In addition to its primary role in the contact activation mechanism of the coagulation system, it releases bradykinin from high molecular weight kininogen which in turn increases permeability of blood vessels and causes other features of the inflammatory reaction (1,2). Plasma kallikrein is also responsible for the generation of fibrinolytic activity (3) and interrelates with the renin-angiotensin system (4). Interaction of plasma kallikrein and

Keywords: complement, coagulation, kallikrein.

complement components might contribute to generation of the inflammatory reaction, since activation of the complement system is an important event in the inflammatory process.

Plasma kallikrein is present in human plasma in an enzymatically inactive form, prekallikrein. Prekallikrein can be converted to kallikrein by the activated form of Factor XII generated by limited proteolysis (5,6). Kallikrein consists of one heavy chain (MW = 43,000) and one light chain (MW = either 33,000 or 36,000). The light chain contains the enzymatically active site (7). The chains, linked by one or more disulfide bonds, can be isolated and their functional properties have been described (8).

It has been reported that kallikrein is capable of cleaving factor B of the alternative pathway of complement (9,10). The cleavage of factor B occurs both in the presence and in the absence of activated C3 (namely C3b) of the complement system (9). Cleavage of factor B has also been reported for other proteolytic enzymes such as trypsin (1) and plasmin (10).

In this paper we have investigated whether cell-bound C3b is also involved in the cleavage of factor B by kallikrein, because this would suggest a role for kallikrein in complement activation at sites of tissue injury. In addition we investigated which chain of kallikrein is involved in activation of B, since it has been reported that the light chain of kallikrein contains the enzymatic active site (7).

MATERIALS AND METHODS

Reagents and antisera. Isotonic Veronal-buffered saline, pH 7.5, containing 0.1% gelatin, 0.5 mM $MgCl_2$ and 0.15 mM $CaCl_2$ (GVB^{++}), half-isotonic GVB^{++} with 3% dextrose ($DGVB^{++}$) and half-isotonic GVB^{++} from which cations were omitted and containing 0.04M EDTA (GVB^{--}-EDTA) were used as diluents for haemolytic assays.

Complement components B (12), $\bar{D}$ (13), C3b (14) and kallikrein (8) and its light chain were purified to homogenity, and the amidolytic activities, assayed as described (8), were comparable. The heavy chain of kallikrein was also prepared from purified kallikrein (8), but it contained 5% contamination of light chain on a molar basis. $\bar{P}$ (15) was functionally purified. $F(ab')_2$ anti human C3 was prepared from goat anti human C3 serum as previously described (14). Human antithrombin III was purified as described (16) and soybean trypsin inhibitor (type 1-S) was purchased from Sigma Chemical Company, St. Louis, MO. Protein concentration was determined by the Folin method (17).

Rat serum was obtained from Wistar rats, bred in the Laboratory of Pathological Chemistry, Leiden. Factor $\bar{D}$ depleted rat serum reagent was prepared essentially as described (18) by gelfiltration of 2 ml normal rat serum on a Sephadex G-75 column (1.5 x 90 cm) using veronal buffered saline with 0.002 M EDTA as elution buffer. The fall-through of the column, devoid of factor $\bar{D}$ haemolytic activity, was pooled. Sheep erythrocytes were from the National Institute of Public Health (Bilthoven, The Netherlands).

B was radiolabeled with ^{125}I to a specific activity of 0.058 µCi/µg protein with the solid phase lactoperoxidase method

(19) and separated from free iodine by gelfiltration on Sephadex G-100.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Cleavage of factor B was studied using SDS-PAGE. Reaction mixtures containing 62 ng ^{125}I-B, constant concentrations of C3b and $\bar{P}$, and varying amounts of $\bar{D}$, kallikrein, light or heavy chain, were applied to 7.5% polyacrylamide gels containing 0.1% SDS. Electrophoresis was carried out at 8 mA/gel and 30°C according to Weber & Osborn (20). After electrophoresis the gels were sliced and the 3 mm slices were counted for radioactivity. Reaction mixtures containing 31 ng ^{125}I-B, constant concentrations of C3b and $\bar{P}$, and varying amounts of $\bar{D}$, kallikrein, light or heavy chain, were applied also to a 9% polyacrylamide slabgel containing 0.07% SDS. Electrophoresis of the 1.5 mm thick gels was carried out at 200 V (21). The gels were stained, destained and dried, before a Fuji X-ray film was applied. After exposure at -70°C the film was developed.

E $F(ab')_2$ C3b cells. 43 µg $F(ab')_2$ fragment of goat IgG2 anti human C3 was added to 400 µl packed sheep erythrocytes in 0.85% NaCl. After dropwise addition of $CrCl_3$ to a final concentration of 24 µM in a buffer containing 0.02M sodium acetate, pH 5.5, in 0.85% NaCl the mixture was kept at room temperature for 10 min. The cells were washed twice with 0.9% NaCl and 350 µg human C3b was added to the packed cells. These were incubated for 30 min at 37°C followed by 30 min at 0°C. After washing the cells twice with 0.9% NaCl and twice with $DGVB^{++}$, the E $F(ab')_2$ C3b were ready for use (14).

Haemolytic assays. To assess formation of cell-bound convertases dilutions of B were incubated with 1 x 10^7 E $F(ab')_2$ C3b, excess (0.5 µg) $\bar{P}$, and $\bar{D}$ for 30 min at 30°C in a final volume of 0.2 ml $DGVB^{++}$. Excess $\bar{P}$ was defined as that amount of $\bar{P}$ which under conditions of limiting additions of B in the presence of $\bar{D}$ induced optimal haemolysis of E $F(ab')_2$ C3b upon further incubation with excess rat serum as developing reagent. The convertase sites generated were developed by further incubation with 0.3 ml rat serum diluted 1:15 in GVB^{--}-EDTA for another 60 min at 37°C. The number of convertase sites (Z) generated were calculated using a reagent blank without B and otherwise identical conditions. There was formation of C3bBb sites in a dose-dependent fashion and directly related to the amount of B added.

To detect the capacity of kallikrein or its light and heavy chain to replace $\bar{D}$, E $F(ab')_2$ C3b were incubated with excess $\bar{P}$ and known amounts of B.

RESULTS

Detection of B activation with haemolytic titrations: Capacity of $\bar{D}$, kallikrein or buffer to activate different concentrations of B and cause the lysis of E $F(ab')_2$ C3b. 1 x 10^7 E $F(ab')_2$ C3b were incubated with excess $\bar{P}$, various concentrations of B and 25 ng (1.1 pmol) $\bar{D}$, 900 ng (11.3 pmol) kallikrein or $DGVB^{++}$ in a final volume of 0.2 ml. Upon addition of rat serum in GVB^{--}-EDTA a dose-dependent lysis of the E

$F(ab')_2$ C3b bearing convertases was observed (fig. 1). Under the conditions used $\bar{D}$ was 380 times more effective then kallikrein on a weight basis. Lysis also occurred without kallikrein or $\bar{D}$, dependent on the dose of B. When we used factor $\bar{D}$ depleted rat reagent to develop the convertase sites generated, this effect was also observed. Using either normal or factor $\bar{D}$ depleted rat reagent, about 60-100 times more B was required to observe the same amount of lysis that occurred in the presence of $\bar{D}$.

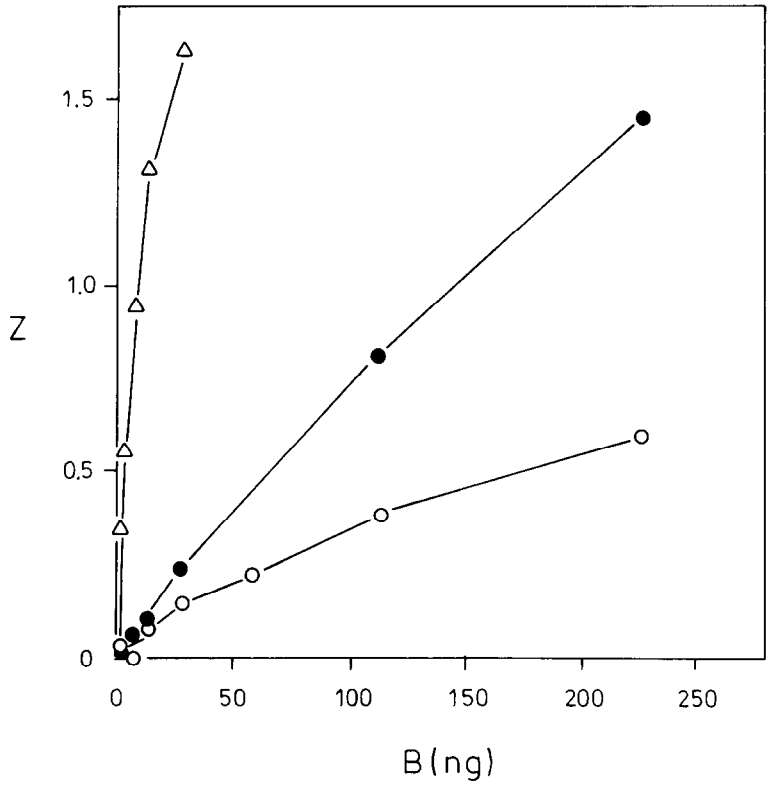

FIG. 1

Dose-response curve for the concentration of B for generation of C3 convertase sites on E $F(ab')_2$ C3b with $\bar{D}$ (Δ), kallikrein (●) or buffer alone (o). 1 x 10^7 E $F(ab')_2$ C3b were incubated with excess $\bar{P}$, various amounts of B and 25 ng $\bar{D}$, 900 ng kallikrein or buffer alone.

Formation of cell-bound C3 convertase sites in the presence of kallikrein and its light and heavy chains. Generation of $\overline{EC3bBb}$ by kallikrein and its light and heavy chains was studied by incubating 1 x 10^7 E $F(ab')_2$ C3b with excess $\bar{P}$, a constant concentration of 19 ng B and various concentrations of kallikrein or its light and heavy chains in a final volume of 0.2 ml

$DGVB^{++}$. The number of C3bBb convertase sites generated with each of the reagents is depicted in fig. 2. There was a dose-dependent increase in the number of convertase sites with kallikrein and its light chain. However on a molar basis the light chain was 35% less active then the intact molecule. The heavy chain of kallikrein was only marginally active. In the same experiment $\bar{D}$ was about 220 times as effective as kallikrein on a molar basis.

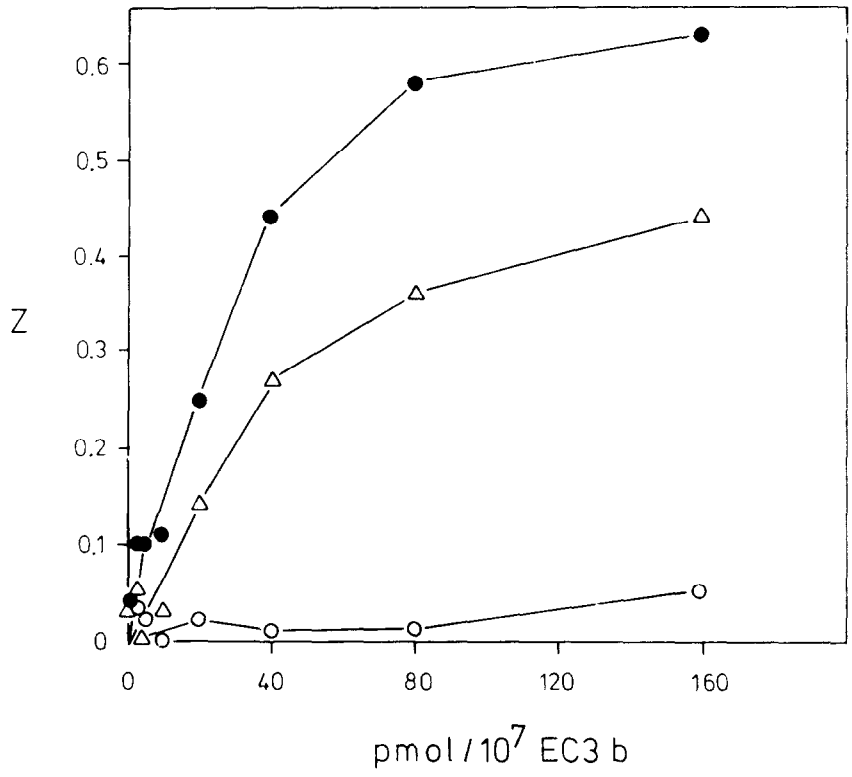

FIG. 2

Formation of cell-bound C3 convertase sites in the presence of kallikrein (●) and its light (Δ) and heavy (o) chains. 1 x 10^7 E $F(ab')_2$ C3b were incubated with excess $\bar{P}$, 19 ng B and various concentrations of kallikrein, light chain and heavy chain.

Consumption of B in the presence of fluid phase C3b by kallikrein and its light and heavy chain. C3b (2.5 µg), B (0.14 µg), kallikrein (0-40 pmoles), light chain (0-40 pmoles), heavy chain (0-40 pmoles) or $DGVB^{++}$ were incubated for 30 min at 37°C in various combinations, diluted at least 40 fold, and assessed for residual haemolytic B activity essentially as described (22). After calculating Z values, the percentage B consumption

was determined. A dose-dependent consumption of B by kallikrein and its light chain was observed, whereas the heavy chain was much less effective (fig. 3). There was no detectable difference between the activities of kallikrein and the light chain. In this experiment $\bar{D}$ was 300 times more effective then kallikrein on a molar basis.

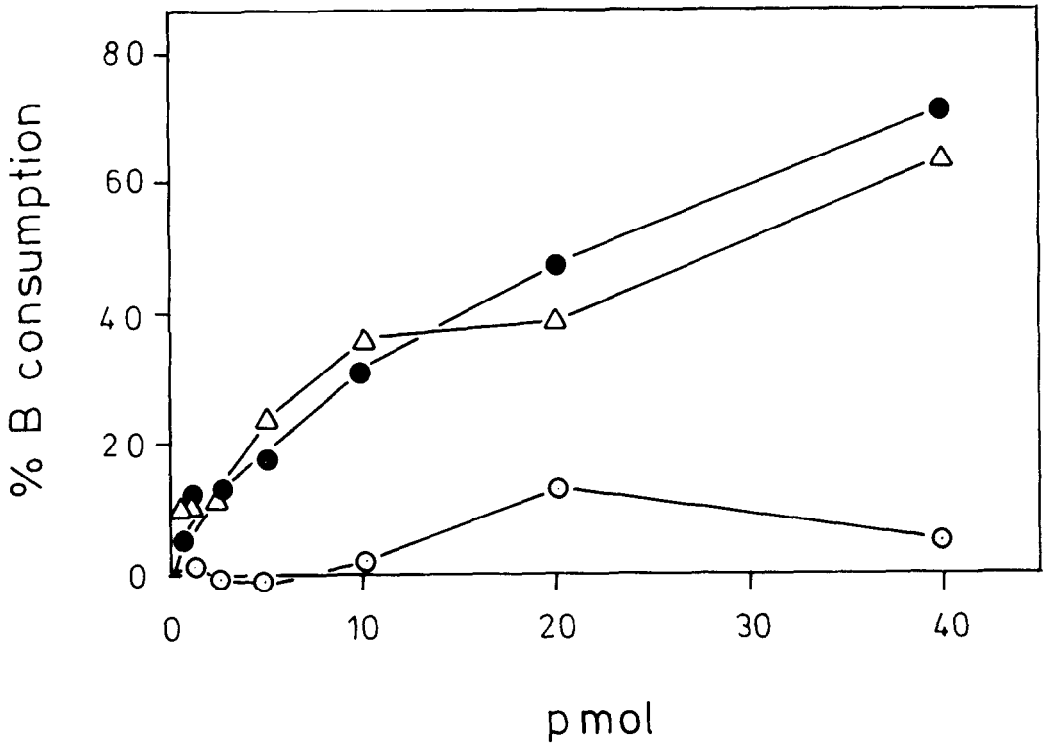

FIG. 3
Consumption of B in the presence of fluid phase C3b by kallikrein (●) and its light (Δ) and heavy (o) chains. The reaction mixtures contained 0.14 µg B, 2.5 µg C3b and various concentrations of kallikrein, light chain and heavy chain.

Inhibition of the consumption of B by kallikrein and its light chain. In order to investigate the effect of soybean trypsin inhibitor and human antithrombin III on the consumption of B by kallikrein and its light chain in the presence of fluid phase C3b, we preincubated 10 pmol kallikrein and its light chain with the inhibitors for 30 minutes at 37°C. Then we added 2.5 µg C3b and 0.14 µg B and incubated the mixtures for another 30 minutes at 37°C and residual haemolytic B activity was assessed in the 100 fold diluted mixtures as described above. The inhibition of the consumption of B by soybean trypsin inhibitor and human antithrombin III is shown in table I.

TABLE I

The effect of preincubation of kallikrein and its light chain with soybean trypsin inhibitor (SBTI) or human antithrombin III (AT III) on the consumption of B in the presence of fluid phase C3b.

2.5 µg C3b and 0.14 µg B was incubated with:	preincubation with (times molar excess over kallikrein or light chain)	inhibition of B consumption (%)
kallikrein	buffer	0
	SBTI 100 x	88
	AT III 10 x	41
light chain	buffer	0
	SBTI 100 x	40
	AT III 10 x	100

Analysis of fragments of ^{125}I-B generated by $\bar{D}$, kallikrein and its light and heavy chains.

In order to investigate whether the size of the fragments of B, generated by kallikrein and its light chain were comparable to those generated by $\bar{D}$, cleavage of ^{125}I-B was studied using SDS-PAGE. 93 ng ^{125}I-B, 32 µg C3b, excess $\bar{P}$ and $\bar{D}$, kallikrein, its light and heavy chains or buffer were incubated in DGVB^{++} in a final volume of 75 µl for 30 min at 37°C. Then 50 µl of the different mixtures was analysed on 7.5% SDS-PAGE gels.

TABLE II

B fragments generated by $\bar{D}$, kallikrein and its light and heavy chains as analysed on sliced gels.

^{125}I-B was incubated with C3b, P and:	pmol	^{125}I-B cleavage (%)
$\bar{D}$	38	95
kallikrein	23	79
kallikrein	113	97
light chain	16	55
light chain	81	93
heavy chain	14	5
heavy chain	72	10
buffer	-	0

* The reaction mixture contained 93 ng ^{125}I-B, excess $\bar{P}$, 32 µg C3b and the indicated amounts of the other agents. These were incubated for 30 min at 37°C and assessed for % B cleavage by SDS-PAGE analysis.

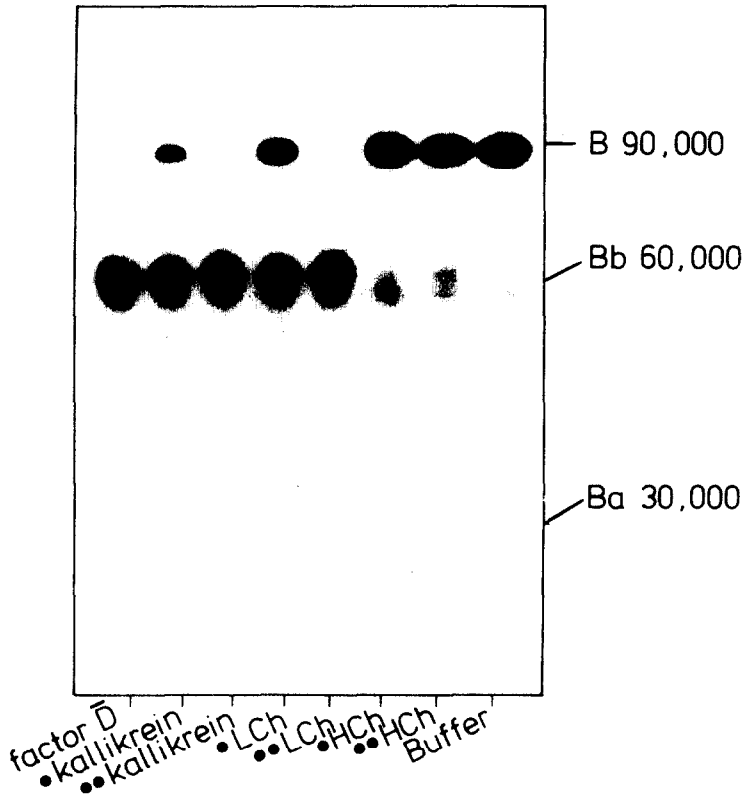

FIG. 4

B fragments generated in reaction mixtures containing C3b, $\bar{P}$ and $\bar{D}$, kallikrein and its light and heavy chains. B fragments generated in reaction mixtures containing 32 µg C3b, excess $\bar{P}$, 93 ng ^{125}I-B and $\bar{D}$ (38 pmol), kallikrein (• : 23 pmol, •• : 113 pmol), its light chain (LCh) (• : 16 pmol, •• : 81 pmol) and heavy chain (HCh) (• : 14 pmol, •• : 72 pmol) or buffer alone, were applied to a 9% SDS-PAGE gel and an autoradiogram was made.

The percentage cleavage was calculated and corrected for the percentage of ^{125}I-B cleavage in a reaction mixture containing C3b, $\bar{P}$ and buffer alone (table II). 25 µl of the different reaction mixtures were also applied to a 9% SDS-PAGE slabgel. After staining, destaining and drying the gels, an autoradiogram was made (fig. 4). The fragments of B generated by $\bar{D}$, kallikrein and its light chain were identical in size (fig. 4). The data in Table II confirm the results shown in fig. 2 and 3: $\bar{D}$ was the most effective in cleaving factor B, kallikrein and its light chain were almost equally effective and no or little cleavage by the heavy chain was observed. In the reaction mixture containing C3b, $\bar{P}$ and buffer alone some B fragments are detectable (fig. 4). This is due to the fact that some cleavage of factor B occurred during the labeling procedure.

DISCUSSION

In this study we demonstrate that kallikrein is capable of cleaving factor B in the presence of fluid phase C3b (fig. 3,4 and table II). These results are at variance with those reported by DiScipio (9). He found cleavage of factor B in the absence of fluid phase C3b. In experiments in which we used up to 14,4 pmol kallikrein to cleave 0.19 µg B we could not demonstrate any cleavage of B by kallikrein in the absence of C3b. Kallikrein is also capable of cleaving factor B in the presence of erythrocyte (E)-bound C3b (fig. 1,2). Lysis of the EC3bBb that were formed indicated that a functionally active C3/C5 convertase was generated. An additional possibility is that kallikrein inactivates cell-bound C3b and by that mechanism may affect the assessment of B hemolytic activity. This is unlikely because others have reported that C3bi is inactivated very slowly by kallikrein (23). The fragments that were found after cleavage of B by kallikrein were indistinguishable in size from those generated by $\bar{D}$, i.e. a Bb fragment of 60,000 and a Ba fragment of 30,000 (fig.4). The capacity of kallikrein to cleave B appeared to be localised in the light chain of the molecule (fig. 2,3,4). The lower activity of the light chain compared to the intact molecule in the presence of E $F(ab')_2$ C3b (fig.2) suggests that the heavy chain plays a role in the adherence of kallikrein to the erythrocyte, comparable to its function in surface-dependent coagulation (8). Addition of high molecular weight kininogen had no enhancing effect on the cleavage of B by kallikrein (unpublished observations). The minor activity of the heavy chain preparation (fig. 2,3) is probably due to contamination with light chain.

In the absence of $\bar{D}$ or kallikrein, functionally active C3 convertase sites were generated with E $F(ab')_2$ C3b, B, $\bar{P}$ and buffer alone (fig. 1). This occurred in a dose-dependent fashion upon addition of B. In our hands we found, in contradistinction with Lesavre and Müller-Eberhard (18) a similar effect using factor $\bar{D}$ depleted rat reagent to develop the convertase sites that were formed. As a matter of fact we cannot exclude minor contamination with $\bar{D}$ of the $\bar{D}$-deficient reagent. On the other hand it is possible that other trypsin-like enzymes may have mimicked $\bar{D}$-like activity. These observations are compatible with previous findings (22,24,25) and indicate that upon incubation of C3, B, and $\bar{P}$ or C3NeF an active C3bB complex can be formed containing an uncleaved form of B that is capable of cleaving C3.

In all our experiments we found that $\bar{D}$ is much more effective in cleaving B than kallikrein. DiScipio (9) concluded that $\bar{D}$ is about ten-fold more effective on a molar basis in the generation of C3 convertase sites than kallikrein. Our experiments indicate that at least 200-300 times more kallikrein than $\bar{D}$ is needed to generate the same number of functionally active C3 convertases. Several pieces of data indicate that these observations are not due to a contamination of kallikrein by $\bar{D}$. DiScipio (9) showed that the effect of kallikrein is inhibitable by soybean trypsin inhibitor and human antithrombin III, as we have also observed (Table I). In the purification procedure of prekallikrein we used a highly specific affinity chromatography step on high molecular weight kininogen (HMWK) Sepharose. Also

the isolated light chain of kallikrein, which does not bind to HMWK Sepharose showed the same effect.

In plasma the concentration of $\bar{D}$ (approx-2µg/ml) (26) is much lower than that of prekallikrein (55 µg/ml) (5) but even if this is taken into account, there is much less C3 convertase generating potential present in prekallikrein then in $\bar{D}$ in plasma. Therefore it is likely that only under those conditions where a high kallikrein concentration is reached, the B cleavage by kallikrein will be of physiological importance. This may occur when a damaged bloodvessel wall causes activation of the contact activation mechanism. Prekallikrein-high molecular weight kininogen complexes then bind to the exposed basal membrane and their subsequent activation by factor XIIa results in the generation of kallikrein. Although the kallikrein concentration rapidly decreases by diffusion and inactivation by inhibitors such as C1 inhibitor (27,28), it might help $\bar{D}$ to generate sufficient numbers of convertase sites to escape inactivation by H and I (29-31).

It has been noted before that other proteins from the contact activation and fibrinolytic systems are able to activate certain complement components. Activated factor XII, βXIIa, was shown to activate the classical pathway of complement in a way similar to immune complexes (32). Plasmin was shown to activate C1 (33) and to cleave B (10). Besides its action on B, plasma kallikrein has been reported to inactivate C1 (33). The interaction of the complement system and the contact activation system may be of physiological importance. Activation of the contact system leads to the activation of the complement system by the action of factor XII_a, kallikrein and indirectly by plasmin. As a result anaphylatoxins are released which attract leukocytes to the site of activation. There the infiltration of the leukocytes into the damaged tissue is facilitated by bradykinin. Bradykinin which increases the vascular permeability is released from high molecular weight kininogen by kallikrein. The infiltrated leukocytes may be important for the clearance of deposited fibrin.

In conclusion kallikrein cleaves B and generates a functionally active C3 convertase. This activity is localised in the light chain of the kallikrein molecule. We therefore conclude that the enzymatic site responsible for the previously described activities is probably also responsible for the cleavage of B. However, whether or not the cleavage of B by kallikrein is of importance for the inflammatory process requires further investigation.

ACKNOWLEDGMENTS

The authors appreciate the excellent technical assistance of Mrs. R.A.A. Vlooswijk. They also acknowledge the secretarial help of Mrs. R. de Graaf.

REFERENCES

1. HABAL, F.M., MOVAT, H.Z. and BURROWES, C.E. Isolation of two functionally different kininogens from human plasma. Separation from proteinase inhibitors and interaction with

plasma kallikrein. Biochem. Pharmacol. 23, 2291-2203, 1974.

2. NAGASAWA, S. and NAKAYASU, T. Human plasma prekallikrein as a protein complex. J. Biochem. (Tokyo) 74, 401-403, 1973.

3. COLMAN, R. Activation of plasminogen by human plasma kallikrein. Biochem. Biophys. Res. Comm. 35, 273-279, 1969.

4. DERKX, F.H.M., BOUMA, B.N., SCHALEKAMP, M.P.A. and SCHALEKAMP, M.A.D.H. An intrinsic factor XII-prekallikrein-dependent pathway activates the human plasma renin-angiotensin system. Nature 280, 315-316, 1979.

5. BOUMA, B.N., MILES, L.A., BERETTA, G. and GRIFFIN, J.H. Human plasma prekallikrein: activation by factor XIIa and inactivation by DFP. Biochemistry 19, 1151-1160, 1980.

6. VAN DER GRAAF, F., KEUS, F.J.A., VLOOSWIJK, R.A.A. and BOUMA, B.N. The contact activation mechanism in human plasma: activation induced by dextran sulfate. Blood 59, 1225-1233, 1982.

7. MANDLE, R. Jr. and KAPLAN, A.P. Hageman factor substrates. Human plasma prekallikrein: mechanism of activation by Hageman factor and participation in Hageman factor-dependent fibrinolysis. J.Biol.Chem. 252, 6097-6104, 1977.

8. VAN DER GRAAF, F., TANS, G., BOUMA, B.N. and GRIFFIN, J.H. Isolation and functional properties of the heavy and light chains of human plasma kallikrein. J.Biol.Chem. 257, 14300-14305, 1982.

9. DISCIPIO, R.G. The activation of the alternative pathway C3 convertase by human plasma kallikrein. Immunology 45, 587-595, 1982.

10. IKARI, N., NIINOBE, M. and FUJII, S. Limited proteolysis of factor B by plasma kallikrein and plasmin. A.A.S. 9, 35-39, 1981.

11. BRADE, V., NICHOLSON, A., BITTER-SUERMANN, D. and HACKLING, U. Formation of the C3-cleaving properdin enzyme on zymosan. Demonstration that factor D is replaceable by proteolitic enzymes. J. Immunol. 113, 1735-1743, 1974.

12. HUNSICKER, L.G., RUDDY, S. and AUSTEN, K.F. Alternate complement pathway: factors involved in cobra venom factor (CoVF) activation of the third component of complement (C3). J.Immunol. 110, 128-138, 1973.

13. DAVIS, A.E. Active site amino acid sequence of human factor D. Proc.nat. Acad.Sci. USA 77, 4938-4942, 1980.

14. DAHA, M.R., BLOEM, A.C. and BALLIEUX, R.E. Immunoglobulin production by human peripheral lymphocytes induced by anti-C3 receptor antibodies. J.Immunol. 132, 1197-1201, 1984.

15. FEARON, D.T. and AUSTEN, K.F. Properdin: binding to C3b and stabilization of the C3b-dependent C3 convertase. J.Exp. Med. 142, 856-863, 1975.

16. DE SWART, C.A.M., NIJMEYER, B., ANDERSSON, L.O., HOLMER, E., SIXMA, J.J. and BOUMA, B.N. Elimination of intraveneously administered radiolabelled antithrombin III and heparin in humans. Thrombos. Haemostas. 52, 66-71, 1984.

17. LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. Protein measurement with the Folin phenol reagent. J.Biol. Chem. 193, 265-275, 1951.

18. LESAVRE, P.H. and MULLER-EBERHARD, H.J. Mechanism of action of factor D of the alternative complement pathway. J. Exp. Med. 148, 1498-1509, 1978.

19. THORELL, J.L. and LARSSON, I. Lactoperoxidase coupled to polyacrylamide for radio-iodination of proteins to high specific activity. Immunochemistry 11, 203-206, 1974.

20. WEBER, K. and OSBORN, M. The reliability of molecular weight determinations by dodecyl sulfate-polycrylamide gel electrophoresis. J.Biol.Chem 244, 4406-4412, 1969.

21. LAEMMLI, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685, 1970.

22. DAHA, M.R., FEARON, D.T. and AUSTEN, K.F. Formation in the presence of C3 nephritic factor (C3NeF) of an alternative pathway C3 convertase containing uncleaved B. Immunology 31, 789-796, 1976.

23. MEUTH, J.L., MORGAN, E.L., DISCIPIO, R.G. and HUGLI, T.E. Suppression of T lymphocyte functions by human C3 fragments. 1. Inhibition of human T cell proliferative responses by a kallikrein cleavage fragment of human iC3b. J. Immunol. 130, 2605-2611, 1983.

24. DAHA, M.R., FEARON, D.T. and AUSTEN, K.F. Isolation of alternative pathway C3 convertase containing uncleaved B and formed in the presence of C3 nephritic factor (C3NeF). J.Immunol. 116, 568-570, 1976.

25. FEARON, D.T. and AUSTEN, K.F. Properdin: initiation of alternative complement pathway. Proc.nat.Acad. Sci.USA 72, 3220-3224, 1975.

26. FEARON, D.T. and AUSTEN, K.F. Activation mechanisms of the alternative complement pathway and amplification step. In: The chemistry and physiology of the human plasma proteins. D.H. Bing (Ed.). New York: Pergamon Press, 1978, pp. 229-254.

27. VAN DER GRAAF, F., KOEDAM, J.A. and BOUMA, B.N. Inactivation of kallikrein in human plasma. J.Clin.Invest. 71,

149-157, 1983.

28. MCCONNELL, D.J. Inhibitors of kallikrein in human plasma. J. Clin. Invest. 51, 1611-1623, 1972.

29. FEARON, D.T. Regulation by membrane sialic acid of β1H-dependent decay dissociation of amplification C3 convertase of the alternative complement pathway. Proc.nat.Acad. Sci USA 75, 1971-1975, 1978.

30. WEILER, J.M., DAHA, M.R., AUSTEN, K.F. and FEARON, D.T. Control of the amplification convertase of complement by the plasma protein β1H. Proc.nat.Acad.Sci. USA 73, 3268-3272, 1976.

31. WHALEY, K. and RUDDY, S. Modulation of the alternative complement pathway by β1H globulin. J.Exp.Med. 144, 1147-1163, 1976.

32. GHEBREHIWET, B., SILVERBERG, M. and KAPLAN, A.P. Activation of the classical pathway of complement by Hageman factor fragment. J.Exp.Med. 153, 665-676, 1981.

33. COOPER, N.R., MILES, L.A. and GRIFFIN, J.H. Effects of plasma kallikrein and plasmin on the first complement component. J.Immunol. 124, 1517, 1980 (abstract).