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Prekallikrein deficiency: The characteristic normalization of the severely prolonged aPTT following increased preincubation time is due to autoactivation of factor XII

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Abstract

Hereditary plasma prekallikrein (PK) deficiency was diagnosed in a 71-year-old man with an 8-year history of osteomyelofibrosis. PK deficiency was suspected in view of a severely prolonged activated partial thromboplastin time (aPTT) that nearly normalized following prolonged preincubation (10 min) of patient plasma with kaolin–inositihin reagent. Hereditary PK deficiency was demonstrated by very low PK values in the propositus (PK clotting activity 5%, PK amidolytic activity 5%, PK antigen 2% of normal plasma, respectively) and half normal PK values in his children. Normalization of a severely increased aPTT (>120 s) after prolonged preincubation with aPTT reagent occurred in plasma deficient in PK but not in plasma deficient in factor XII (FXII), high-molecular-weight kininogen (HK), factor XI (FXI), factor IX, factor VIII, Passovoy trait plasma or plasma containing lupus anticoagulant. Autoactivation of FXII in PK-deficient plasma in the presence of kaolin paralleled the normalization of aPTT. Addition of OT-2, a monoclonal antibody inhibiting activated FXII, prevented the normalization of aPTT. We conclude that the normalization of a severely prolonged aPTT upon increased preincubation time (PIT), characteristic of PK deficiency, is due to FXII autoactivation.

Keywords: Activated partial thromboplastin time (aPTT); Prekallikrein deficiency; Factor XII autoactivation; Prekallikrein autoactivation; Preincubation time; Contact phase system

1. Introduction

The activated partial thromboplastin time (aPTT) is a coagulation test that encompasses all steps of the intrinsic pathway of blood coagulation from the activation of the contact phase system to fibrin formation. In this test, an aliquot of citrated plasma is preincubated with an activator of the contact phase system and a so-called partial thromboplastin. Kaolin, celite and ellagic acid are used as activators (providing the negatively charged surface) for the contact phase system. Inositihin, cephalin or other negatively charged phospholipids serve as a "partial thromboplastin" providing platelet factor 3-like activity. During the preincubation of plasma with the aPTT reagent (mixture of activating "surface" and partial thromboplastin), the contact phase system is activated. The contact system is comprised of three serine protease zymogens, factor XII (FXII; M_r 80,000, plasma concentration 24–40 μg/ml), prekallikrein (PK; M_r 80,000, 50 μg/ml) [1,2] and factor XI (FXI; M_r 160,000, 4–8 μg/ml) as well as the nonenzymatic cofactor high-molecular-weight kininogen (HK; M_r 120,000, 70–80 μg/ml). Its activation leads to the formation of factor XIIa (FXIIa), kallikrein (KK) and factor XIa (FXIa) [3,4]. After the preincubation phase, plasma is recalcified and the clotting time is measured. Deficiencies of contact activation factors, intrinsic or common pathway factors as well as the

Abbreviations: FXIa and FXIIa, activated coagulation factors XI and XII; aPTT, activated partial thromboplastin time; AR, autoradiography; BA, barbiturate acetate; BSA, bovine serum albumin; FVIII, coagulation factor VIII; FIX, coagulation factor IX; FXI, coagulation factor XI; FXII, coagulation factor XII; CAT, contact activation time; HK, high-molecular-weight kininogen; IB, immunoblot; KK, kallikrein; min, minute; NC, negative control; NHP, normal human plasma; PIT, preincubation time; PK, prekallikrein; s, second; SDS, sodium dodecyl sulfate.

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presence of inhibitors such as lupus anticoagulant or heparin lead to prolongation of the aPTT.

In this report, we describe a new case of hereditary PK deficiency in a 71-year-old man with an 8-year history of osteomyelofibrosis. We studied plasmas from patients with various conditions associated with a prolonged aPTT, in order to confirm that normalization of aPTT following prolonged preincubation was specific for PK deficiency and to elucidate the mechanism underlying this normalization.

2. Materials and methods

2.1. Plasma sampling

Citrated blood samples were obtained by clean venipuncture using 19-gauge butterfly needles and Monovette tubes (Sarstedt, Nümbrecht, Germany) containing 1/9 vol of 0.106 M Na_3 citrate. Plasma was prepared by double centrifugation (1500 × *g*, each for 10 min, at room temperature) and was stored in polypropylene tubes at −70 °C until use.

2.2. Reagents

Pooled normal human plasma (NHP) of 42 healthy male volunteers was used as a standard and defined to contain 100% of clotting factor activity and antigen, respectively. For aPTT experiments a plasma pool of seven healthy male volunteers was used as a control (control plasma, Fig. 2a). Plasmas congenitally deficient in PK, HK, FXII, FXI and Passovoy trait plasma [5,6] were purchased from George King Biomedical (Overland Parks, KS) (GK1703, GK 1603, GK 1205, GK 1111 and GK 1501). Lupus anticoagulant plasma and plasmas deficient in FVIII or FIX were obtained from patients of our outpatient ward. All further reagents used were of reagent grade.

2.3. Protein purification and radiolabelling

PK was isolated according to van der Graaf et al. [7] with a minor modification [8]. HK was purified according to Kerbiriou and Griffin [9], FXI according to Bouma and Griffin [10] and FXII according to Griffin and Cochrane [11]. The isolated proteins were kept in 4 mM sodium acetate, 2 mM acetic acid, 150 mM NaCl at pH 5.3 and stored at −70 °C.

Radiolabelling of PK, HK, FXII and FXI was performed with ^{125}Iodine (^{125}I; IMS 30 Amersham International, Buckinghamshire, UK) using the chloramine T method [12]. Specific radioactivity was 27 μCi/μg for PK, 81 μCi/μg for HK, 29 μCi/μg for FXII, as well as 61 μCi/μg for FXI. The radiolabelled proteins were stored in a buffer containing 50 mM sodium acetate, 140 mM sodium chloride, 3 mM NaN_3 and 10 mg/ml bovine serum albumin (BSA), pH 5.2, at −70 °C.

2.4. Clotting activity tests and contact factor assays

aPTT was assayed using a tilt tube technique. A kaolin–inositihin suspension (10 mg/ml kaolin, 0.5 mg/ml inositihin) in isotonic barbiturate acetate (BA) buffer, pH 7.35, was used as the aPTT reagent. One hundred microliters of prewarmed plasma and 100 μl of kaolin–inositihin suspension were preincubated in a glass tube at 37 °C. After preincubation [standard preincubation time (PIT) = 1 min; for activation experiments, this time varied from 1 to 180 min], 100 μl of prewarmed (37 °C) 0.025 M $CaCl_2$ was added and the clotting time was recorded in seconds.

PK, HK and FXII clotting activity (PK:C, HK:C and FXII:C) were measured as previously described [13]. Briefly, 50 μl of the respective deficient plasma was mixed with 50-μl samples of test plasma, diluted at least 1:10 in BA buffer containing 1 mg/ml BSA, and 50 μl of Neothromtin (Dade Behring, Marburg, Germany) and incubated at 37 °C. After preincubating the mixture for 1 min (PK:C) or 4 min (HK:C, FXII:C), respectively, 50 μl of 0.025 M $CaCl_2$ was added and clotting time was measured.

PK amidolytic activity (PK:Am) was assayed photometrically using a commercial kit, Coaset PK (Chromogenix, Mölndal, Sweden).

To assay PK antigen (PK:Ag), autoradiographs of immunoblots were scanned using a personal laser densitometer (Molecular Dynamics, Sunnyvale, CA) and a commercial software (MD Image Quant, Version 3.3). The result of this scanning procedure, termed "volume," is dependent on the quantity of antigen present in any band. The volume values of NHP dilutions were used to produce a calibration curve of PK:Ag (in percent of NHP) vs. volume as depicted in Fig. 1.

The PK calibration curve was calculated using Table Curve 2D for Windows (Jandel Scientific Software, San Rafael, CA; Version 3) and a transition function [logistic dose response curve: $y = a/(1+(x/b)^c]$ to fit the data.

2.5. Contact activation of plasma

Kaolin was suspended in isotonic BA buffer, pH 7.35 (kaolin 10 ×: 100 mg/ml). Nine volumes of prewarmed plasma and one volume of kaolin suspension were incubated at 37 °C for 1, 2, 5, 10, 15, 30, 60, 120 and 180 min, respectively. For nonactivated samples, kaolin suspension was replaced by BA buffer. To terminate activation, reaction mixtures were diluted in 0.01 M Tris, 0.14 M NaCl, pH 7.4 (TBS) containing 1 mg/ml BSA and 100 μg/ml soy bean trypsin inhibitor (SBTI) and immediately boiled for 5 min in sample buffer containing sodium dodecyl sulfate (SDS).

2.6. SDS polyacrylamide gel electrophoresis (PAGE)

Plasma samples were subjected to SDS-PAGE [14] on slab gels under either reducing (HK, FXI and FXII activation assays) or nonreducing conditions (PK antigen meas-

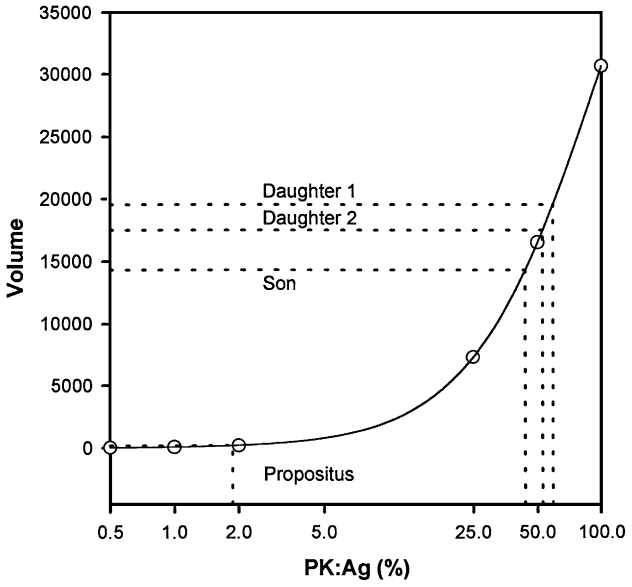

Fig. 1. Quantitative immunoblotting of PK in plasma from the propositus, his son and two daughters following SDS-PAGE. The graph shows the calibration values (open circles: 0.01, 0.02, 0.04, 0.50, 1.0 and 2.0 μl of NHP) and the curve-fitted calibration curve (logistic dose response curve; r^2=.999). Dashed lines connect the observed "volume" values of the four persons studied and the derived values for PK:Ag in percent.

urement). Samples run under reducing conditions had been reduced and alkylated using dithio-D,L-threitol (DTT) and iodoacetamide (IAA). 7.5% (wt/vol of acrylamide) gels was used for PK detection and 10% gels for HK, FXII and FXI assays. Bisacrylamide was added to make up 2.6% (wt/wt) of total monomer. In all experiments, the first and last lanes were utilized for negative controls (NC) and prestained protein standards (BioRad, Hercules, CA), respectively.

2.7. Immunoblotting and autoradiography (AR)

For the quantification of PK:Ag and the detection of FXII, HK and FXI activation, the electrophoretically separated proteins were blotted onto nitrocellulose membranes (Protran BA 83, 0.2 μm; Schleicher and Schüll, Dassel, Germany). Following blocking of unspecific binding sites using a 5% (wt/vol) solution of milk powder [15], the membrane-bound PK, HK and FXII were detected using appropriate polyclonal antisera and subsequent overlay with ^{125}I-labelled PK, HK and FXII, respectively [16]. Polyclonal goat antisera directed against PK and FXII were a kind gift of Prof. JH Griffin (Scripps Research Institute, La Jolla, CA). The anti-PK antiserum recognizes the heavy and light chains of PK but can only be used for proteins separated under non-reducing conditions [13,16]. The FXII antiserum, on the other hand, can be used for detection of reduced FXII, but then will almost exclusively detect the heavy chain [16]. The rabbit polyclonal anti-HK antiserum was directed against the reduced and alkylated 47-kDa light chain [17].

AR was performed following immunoblotting procedures (for the PK, HK and FXII assays) or immediately after the transfer onto the nitrocellulose sheet (for ^{125}I–FXI activation experiments) by exposing X-ray film (Cronex 4, medical x-ray film, Dupont, Bravard, NC) in cassettes lined with intensifier foils (MR 200 or MR 400; Agfa Gevaert, Schrobenhausen, Germany). Exposure times varied from 3 to 48 h depending on the assay, the antiserum dilution and the specific activity of the radiolabelled protein.

For immunoblotting and AR experiments (Fig. 3), 1 μl of plasma was applied per lane (lanes 1–11: labelled NC, 0, 1, 2, etc.). The first lane of each gel was reserved for a NC: 1 μl of FXII-deficient and HK-deficient plasma or 1 μl of ^{125}I–FXI free plasma were loaded in blots of rows A, B and C, respectively. Plasma samples were either not activated (0 min) or activated for varying times (1, 2, 5, 10, 15, 30, 60, 120 and 180 min). The horizontal bars depicted in the last lane indicate the position of molecular weight standards with their respective M_r listed to the right.

3. Results

3.1. Case presentation

The propositus, a 71-year-old Caucasian man with an 8-year history of idiopathic osteomyelofibrosis, was hospitalized for a planned splenectomy. The splenectomy became necessary because of thrombocytopenia and anemia necessitating frequent transfusions. Osteomyelofibrosis had been diagnosed following symptoms of anemia. No bleeding or thrombotic events were known in the propositus and his family. The propositus had three healthy children, one son and two daughters.

During routine preoperative screening, a prolonged aPTT of 422 s (normal range 40–60 s) was observed while the thromboplastin time (prothrombin time according to Quick)

Table 1
Results of coagulation tests in the propositus, his son and two daughters

Sample	aPTT (40–60 s)	Quick (70–130%)	PK:C (65–120%)	PK:Am (65–120%)	PK:Ag (nd)	FXII:C (61–145%)
Propositus	422	73	5	5	2	54
Son	51	77	48	58	44	nd
Daughter 1	56	96	58	69	59	nd
Daughter 2	50	95	54	62	53	nd

aPTT, activated partial thromboplastin time; Quick, thromboplastin time; PK, prekallikrein; PK:C, PK clotting activity; PK:Am, PK amidolytic activity; PK:Ag, PK antigen; FXII:C, factor XII clotting activity; nd, not determined; the normal range of the respective assay is given in parentheses.

and thrombin time were within normal limits. Mixing equal volumes of NHP and propositus plasma led to a normal aPTT indicating a coagulation factor deficiency and excluding a plasmatic inhibitor of the intrinsic system. When the PIT of plasma with the aPTT reagent was prolonged to 10 min, the resulting aPTT was 71 s (normal 40–60 s). Therefore, PK deficiency was suspected.

3.2. Quantitative immunoblotting of PK and coagulation studies in the propositus and his family

Fig. 1 shows the calibration curve used to determine PK:Ag values of the propositus and his family. "Volume" values indicative of PK:Ag present were measured for various NHP dilutions (0.01, 0.02, 0.04, 0.50, 1.0 and 2.0 μl of NHP, open circles). Using these values, a calibration curve was generated from which the plasma PK content in percent of NHP was determined. The results for PK:Ag as well as those of other coagulation assays are listed in Table 1.

3.3. Effect of varying PITs on aPTT in various plasmas

The aPTT was measured using variable PITs in plasma samples from patients with conditions associated with prolonged aPTT. Fig. 2a shows the normalization of aPTT in PK-deficient plasma (George King Biomedical) with increasing PIT. With both normal control plasma and PK-deficient plasma, we observed shortening of the aPTT with PITs increasing from 1 min up to 10–20 min. Further prolongation of the PIT slightly increased clotting times. The aPTT values of the pooled control plasma were within the normal range of 40–60 s at PITs from 1 min up to 60 min.

Addition of the monoclonal antibody OT-2 directed against FXIIa at a final concentration of 50 μg/ml after 5-min preincubation not only abolished the normalization of aPTT expected after 20-min PIT but led to a drastically prolonged aPTT value at 20 and 60 min PIT as compared to control (BA buffer instead of antibody OT-2 added to PK-deficient plasma at 5 min of preincubation).

aPTT vs. PIT of plasmas deficient in contact phase proteins (PK-deficient propositus plasma, plasmas deficient in FXII, HK or FXI) is shown in Fig. 2b, and of plasmas

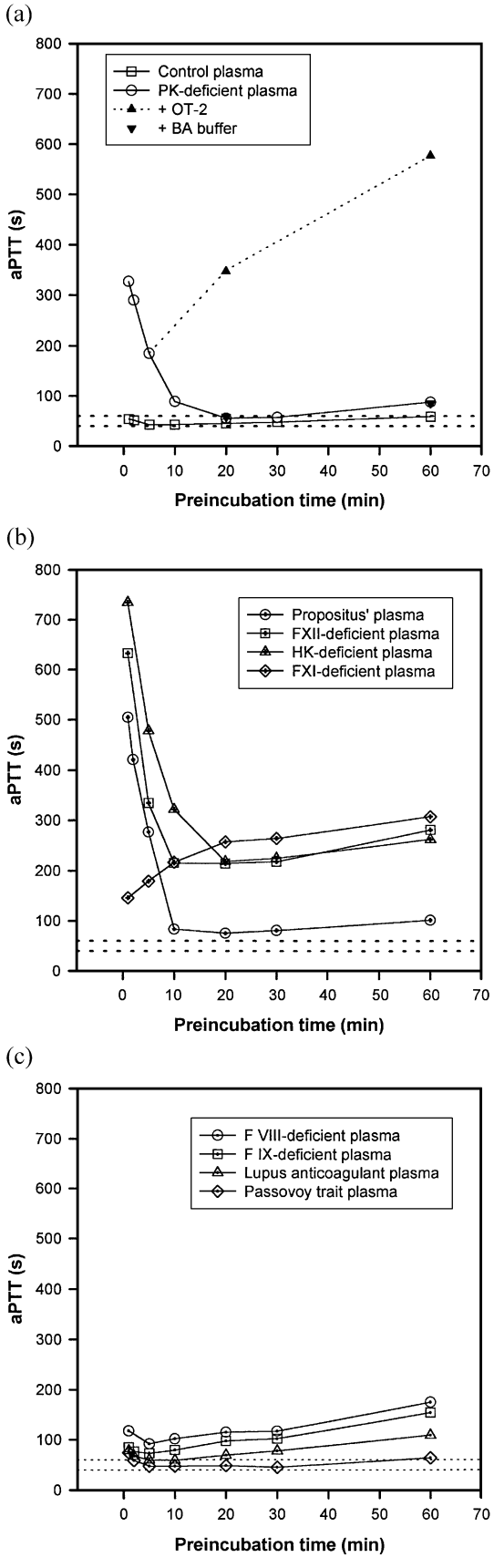

Fig. 2. aPTT vs. PIT in various plasmas with a prolonged aPTT. Each point represents the arithmetic mean of duplicate measurements. The aPTT was measured after 1, 2, 5, 10, 20, 30 and 60 min of PIT. The normal aPTT range is 40–60 s using 1-min PIT (horizontal dotted reference lines). The results from normal control plasma as well as PK-deficient plasma (George King Biomedical) are depicted in (a). When 20 μl of the FXIIa-specific monoclonal antibody OT-2 (final concentration 50 μg/ml) was added to the preincubation mixture after 5 min of PIT, the resulting aPTT after 20 and 60 min were greatly prolonged (solid triangles with base facing down) as compared to control (20 μl of BA buffer added after 5 min; solid triangles with base facing up). Results for PK-deficient propositus', FXII-deficient, HK-deficient and FXI-deficient plasmas (b) as well as FVIII-deficient, FIX-deficient, lupus anticoagulant and Passovoy trait plasmas (c) are shown for comparison.

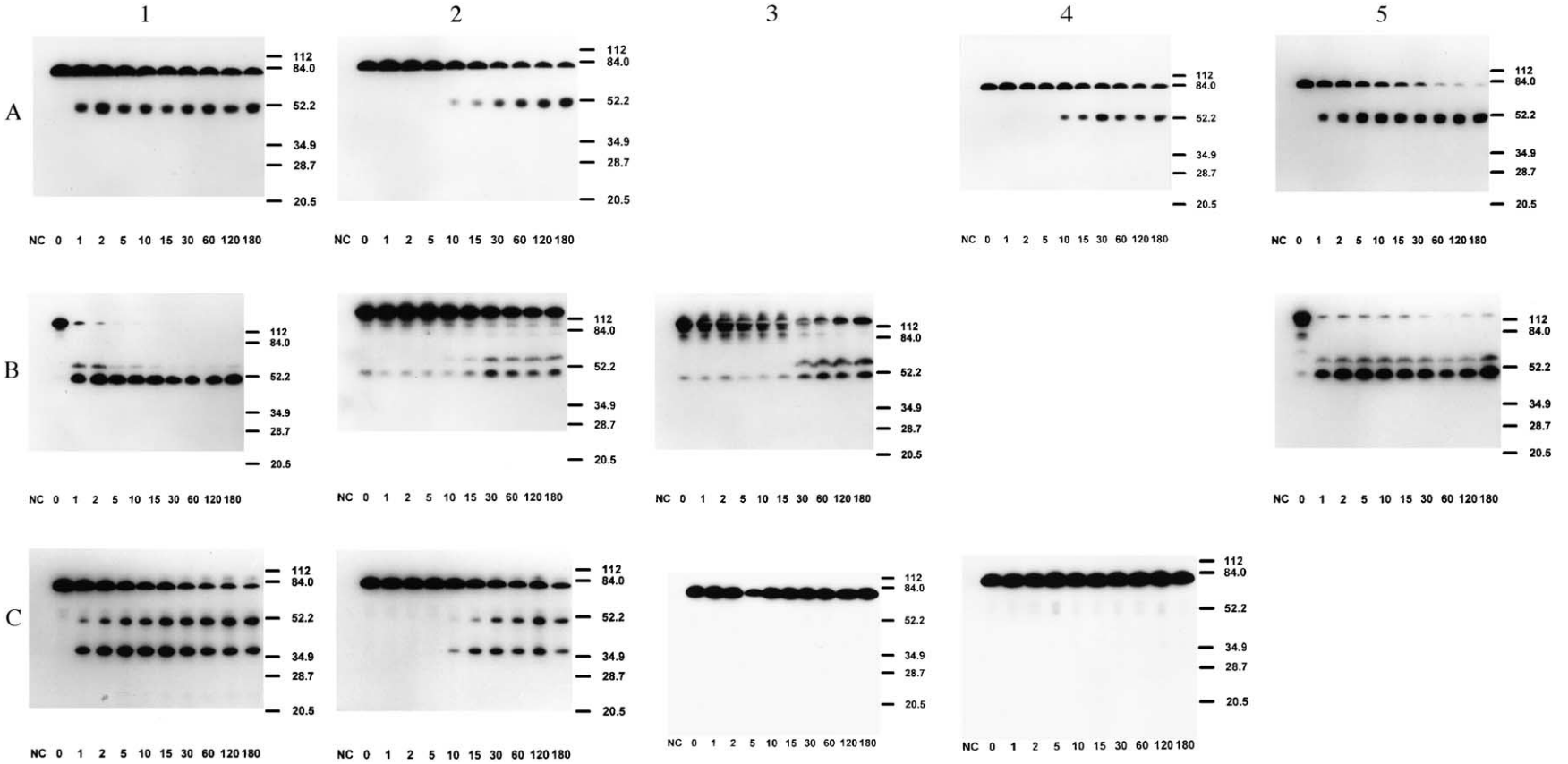

Fig. 3. FXII (row A) and HK immunoblots (row B) and ^{125}I–FXI autoradiographies (row C) of NHP (column 1) as well as PK-deficient (column 2), FXII-deficient (column 3), HK-deficient (column 4) and FXI-deficient plasma (column 5) activated by kaolin (10 mg/ml). The goat anti-FXII antiserum used in FXII IB detects the 80-kDa uncleaved FXII molecule as well as the 52-kDa heavy chain [16]. For HK IB, a rabbit antiserum against the reduced 47-kDa light chain of HK [17] was used, leading to the detection of 120-kDa uncleaved HK, cleaved 61-kDa light chain and cleaved light chain at 47 kDa. The autoradiographies with ^{125}I-radiolabelled FXI, on the other hand, permitted detection of the uncleaved 80-kDa monomeric form of the protein as well as the 50- and 30-kDa heavy and light chains, respectively.

deficient in FVIII and FIX and of Passovoy trait plasma (George King Biomedical) as well as plasma containing lupus anticoagulant in Fig. 2c. The graphs of FXII- and HK-deficient plasmas showed a time course of aPTT, which was similar to that of PK-deficient plasma but with a higher plateau value, i.e., there was no normalization of the aPTT values. The propositus' plasma showed a pronounced shortening of the aPTT without complete normalization. The graph of FXI-deficient plasma had a remarkable time course, as it was the only example with a continuously rising aPTT with increasing PIT. aPTT curves for FVIII- and FIX-deficient plasmas showed a trend toward normalization after 5-min PIT, but differed from the curve for PK-deficient plasma by shorter initial aPTT and lack of actual normalization. The plots for lupus anticoagulant and Passovoy trait plasmas both showed a normalization of aPTT after 5–10 min of PIT (Fig. 2c), but the standard aPTT (with 1-min PIT) was only moderately increased (aPTT < 120 s).

3.4. Kaolin mediated activation of contact phase proteins in NHP and contact factor-deficient plasmas

The results of FXII, HK and FXI activation experiments in NHP, PK-deficient plasma, FXII-deficient plasma, HK-deficient plasma and FXI-deficient plasma are shown in Fig. 3. In NHP, cleavage of FXII, HK and FXI was detectable after 1 min of contact activation time (CAT; Fig. 3, blots A1, B1 and C1). On the other hand, in PK-deficient plasma, the proteolysis of these contact phase proteins occurred after 10-min CAT (Fig. 3A2, B2 and C2). Studies with FXII-deficient plasma showed HK cleavage after 30-min CAT (Fig. 3B3) but no FXI cleavage after 60-min CAT (Fig. 3C3). In HK-deficient plasma, FXII cleavage was present after 10-min CAT (Fig. 3A4) but no FXI cleavage was detected after 60 min (Fig. 3C4). In FXI-deficient plasma, FXII and HK activation occurred after 1-min CAT (Fig. 3A5 and B5) as in NHP.

4. Discussion

The normalization of aPTT following prolonged PIT was first described in PK deficiency by two groups of authors in 1970 [18,19]. A Medline literature search using "PTT" and "incubation time" as medical subject headings (PTT is the designated MESH to be used instead of aPTT) identified only two other pertinent papers, both dealing with lupus anticoagulants. The first paper demonstrates a shortening of aPTT by more than 11 s following prolonged incubation time in 40 lupus anticoagulant plasmas; but it is noteworthy that using the respective assay conditions, none of the studied plasmas showed aPTT normalization [20]. The second study partially confirms the utility of aPTT shortening following prolonged PIT as a diagnostic test for lupus anticoagulants using three different activating agents but does not provide information regarding aPTT normalization [21]. Our coagulation studies using a wide range of plasmas associated with prolonged aPTT confirm that the normalization of a severely prolonged aPTT (>120 s) following prolonged PIT only occurs in PK-deficient plasma. There were two other plasmas, Passovoy trait plasma and lupus anticoagulant plasma, that also showed a normalization of aPTT. The extent of standard aPTT (1-min PIT) prolongation (< 80 s vs. >300 s for PK-deficient plasma) and the ratio of standard aPTT/minimum aPTT (< 2:1 vs. 6:1 for PK-deficient plasma) distinguish the former two plasmas from PK-deficient plasma. In the literature, severe PK deficiencies with aPTT values shorter than 120 s have been described. This has to be ascribed to differences in activating agents and length of PIT [22–24]. In our assay system, we used kaolin as contact-activating substance, inositin as partial thromboplastin and a standard PIT of 1 min.

Several cases of hereditary PK deficiency have previously been described [24], many of them in association or coexisting with other conditions, such as frost bite [1], epistaxis [25], dental problems [26], Graves' disease [27,28], congestive heart failure [23], myocardial infarction [29], tuberculosis [19], hemorrhoids [24], rectal bleeding [19], uterus myomatosus [30,31], prostate hyperplasia [23], fractured wrist [19], hemarthrosis [32], shoulder pain [13], congenital arthrogryposis [33], stroke [34], multiple cerebral infarctions [35] and chronic lymphocytic leukemia [36]. In most cases, however, PK deficiency was diagnosed during routine screening. Hence, a coexisting disease leading to coagulation screening may be necessary for the discovery of PK deficiency, but the association of the respective condition with PK deficiency should not be confounded with causality.

The new case of hereditary PK deficiency we describe was discovered following routine preoperative coagulation studies, which had shown a severely prolonged aPTT in a patient with coexisting osteomyelofibrosis. The man had no history of hemorrhagic or thrombotic events and splenectomy was performed without complications. Although only two generations were available for testing, the mode of inheritance is compatible with an autosomal recessive trait. The patient, being double heterozygous or homozygous, had a severely prolonged aPTT and PK antigen level of 2%. His son and two daughters, putatively being heterozygous, had normal aPTT and PK:Ag of 44%, 53% and 59%, respectively. The family is of Caucasian origin; consanguinity was not assessed. The absence of complete aPTT normalization of the propositus' plasma can be attributed to a concurrent mild deficiency of FXII (Table 1).

The immunoblotting and AR studies demonstrate the processes underlying contact system activation in various plasmas. In NHP, reciprocal activation of FXII and PK occurred, leading to formation of FXIIa and KK. KK in turn cleaves HK and FXIIa activates FXI. The amount of FXIa generated during preincubation influences the extent of FIX activation after recalcification and, thereby, determines the clotting time [3]. In fact, in NHP, the shortening

of aPTT (as a function of PIT) in the coagulation studies correlates well with the activation or cleavage of FXI (as a function of CAT) in the AR studies.

The mechanism responsible for normalization of aPTT in PK deficiency is illustrated by the immunoblotting and AR experiments in PK-deficient plasma (Fig. 3, column 2). Autoactivation of FXII to FXIIa is detected after 10-min CAT (Fig. 3A2). This autoactivation precedes the production of FXIa, apparent after 10–15 min of CAT (Fig. 3C2). The generation of FXIa in the activation studies correlates with the normalization of aPTT in the clotting studies. The hypothesis of FXII autoactivation is supported by the OT-2 experiments discussed below. HK cleavage observed in the absence of PK (Fig. 3B2) may be due to FXIIa- or FXIa-induced proteolysis, both having been previously described [37–39].

The principle of FXII autoactivation, i.e., FXII activation by FXIIa, was postulated ever since the normalization of aPTT in PK-deficient plasma was described [18]. This hypothesis was corroborated by demonstrating cleavage of glass-bound FXII initially by PK-deficient plasma [40] and later by isolated FXIIa [41]. The related concept (based on experiments performed with purified proteins)—that in whole plasma, KK-mediated FXII activation occurs much faster and hence predominates over autoactivation of FXII whenever KK is present [42]—is supported by our results obtained in plasma.

To prove the role of FXII autoactivation in aPTT normalization upon prolonged PIT of PK-deficient plasma, we repeated coagulation studies using OT-2, a monoclonal antibody directed against the active site of FXIIa [43], which we added to the preincubation mixture after 5-min preincubation (Fig. 2a). As a result of the equal amounts of FXIa generated during the first 5 min of PIT, one could expect that the resulting aPTT after addition of OT-2 (with recalcification at 20 or 60 min) would be equal to that observed after 5-min PIT. In fact, we observed not only an inhibition of further shortening but a prolongation of aPTT following the addition of OT-2. We speculate that the small amounts of FXIa generated during the first 5 min of preincubation are progressively inhibited by serpins, such as C1 inhibitor [44], during the remaining 15 or 55 min, respectively, preceding recalcification. This phenomenon is illustrated by blots C1 and C2 of Fig. 3 showing a faint band at approximately 110 kDa appearing after 120-min CAT. This band most likely corresponds to C1 inhibitor–FXIa light chain complex.

Immunoblotting studies of FXII-deficient plasma show cleavage of HK after 30-min CAT (Fig. 3B3) in the absence of FXI activation. The resulting HK light chains of 61 and 47 kDa suggest that this proteolysis is due to PK autoactivation, which has previously been described using purified PK [45]. No FXI activation occurs in FXII-deficient plasma (Fig. 3C3).

The results of experiments with HK-deficient plasma show that in the absence of HK, FXII activation occurs (Fig. 3A4), whereas FXI remains uncleaved (Fig. 3C4). FXII activation is slightly faster than the autoactivation witnessed in PK-deficient plasma (Fig. 3A2) and this is likely due to the presence of free KK. Thus, the absence of HK as a cofactor does not completely prevent reciprocal FXII and PK activation. However, the presence of this cofactor seems necessary for FXIIa-induced FXI activation, as shown by the lack of FXI activation in autoradiograph C4. The fact that in the absence of detectable FXI activation the aPTT still shortens considerably with increasing PIT (1–20 min, Fig. 2) in FXII- as well as in HK-deficient plasmas remains unexplained. This may be due to generation of traces of FXIa not detectable in our assay. Alternatively, a FXI-independent pathway relating the contact phase system to FIX or any other coagulation factor further downstream may exist.

In FXI-deficient plasma, FXII activation (Fig. 3A5) and HK cleavage (Fig. 3B5) occur similarly as in NHP. The seemingly unexplained formation of a fibrin clot (rendering possible the measurement of the aPTT) in FXI-deficient plasma, as illustrated in Fig. 2b, supports the hypothesis of a FXI-independent pathway mentioned above.

In conclusion, the coagulation studies, in conjunction with the immunoblotting and AR experiments—performed in a whole plasma system—show that the normalization of a severely prolonged aPTT following prolonged PIT, pathognomonic for PK-deficient plasma, is due to autoactivation of FXII. Our coagulation data on FXII- and HK-deficient plasmas furthermore suggest a putative FXI-independent link between FXII or PK and downstream elements of the coagulation cascade.

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