原 著

第Ⅷ因子インヒビター作用を持たない抗第Ⅷ因子モノクローナル抗体を用いた第Ⅷ因子抗原測定法 (ELISA)

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Enzyme-linked immunosorbent assay (ELISA) of factor VIII antigen using a monoclonal antibody devoid of factor VIII inhibitor activity

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純化第Ⅷ因子を免疫した Balb/C マウス脾細胞と X-63 ミエローマ細胞とを細胞融合させることによって1種類の抗第Ⅷ因子モノクローナル抗体を得た。抗体の検出は4相からなる immunoradiometric assay (IRMA) によった。本モノクローナル抗体(NMC-VIII/1)の抗体力価は IRMA 法にて 1×10^6 倍であったが、第Ⅷ因子凝固活性 (F.VIII:C) 抑制作用は認められなかった。免疫グロブリンサブクラスは IgG₁ であった。本モノクローナル抗体を固相化した免疫吸着体により、F.VIII あるいは F.VIII/vWF 複合体が吸着された。次に、本抗体を用いた固相—サンドイッチ ELISA 法による第Ⅷ因子抗原 (F.VIII:Ag) の測定系を確立した。本 ELISA 法により、正常血漿、正常血清、および、血友病 A 患者血漿サンプルの F.VIII:Ag を測定し、同時にポリクローナルな高力価同種抗体を用いた IRMA 法による F.VIII:Ag 値と、凝固一段法による F.VIII:C 値とを比較検討した。正常血漿、および、血友病 A 血漿サンプルでは、モノクローナル ELISA による F.VIII:Ag 測定値はポリクローナル IRMA による測定値とよく一致した。また、5 例の中等〜軽症型患者血漿においていずれの方法によっても有意な F.VIII:Ag が検出された。一方、正常血清サンプルにおける F.VIII:Ag の平均値は 14 ± 5 u/dl でポリクローナル IRMA による測定値よ

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りはるかに低値を示した。この F.VIII:Ag の低下は凝固開始後直ちに認められた。

Abstract

To obtain a monoclonal antibody to F.VIII, we immunized BALB/c mice with purified F.VIII and fused the spleen cells with X-63 murine myeloma cells. The hybridomas were screened for F.VIII antibody production by four-layer IRMA. Consequently one hybridoma was cloned which secreted F.VIII monoclonal antibody (NMC-VIII/1) detectable by IRMA but lacking inhibitory activity to F.VIII:C. Mouse ascitic fluid produced by the hybridoma cells had an antibody titer of 1×10^5 . The immunoglobulin was of IgG₁ subclass. Immuno-adsorbent beads coupled with the monoclonal antibody adsorbed only F.VIII and F.VIII/vWF. Two-site solid phase ELISA for F.VIII:Ag was developed using the monoclonal antibody. F.VIII:Ag in normal plasma, normal serum and hemophilia A plasma was assayed by this method (F.VIII:Ag ELISA), and the results were compared with F.VIII:Ag values measured by ELISA using homologous polyclonal antibodies (F.VIII:Ag IRMA) and with F.VIII:C values measured by one-stage clotting assay. In normal and hemophilic plasmas, F.VIII:Ag ELISA levels correlated well with F.VIII:Ag IRMA. A significant level of F.VIII:Ag was detected equally by both methods in 5 moderate to mild hemophiliacs. In normal sera, the average value of F.VIII:Ag ELISA was 14 ± 5 u/dL, which was much lower than F.VIII:Ag IRMA. The reduction of F.VIII:Ag ELISA occurred rapidly after the beginning of blood clotting.

Introduction

It is very difficult to investigate the molecular nature of F.VIII because of its low content in plasma, poor stability and existence as complexes with von Willebrand factor. Immunochemical studies of F.VIII have relied chiefly on the availability of human F.VIII antibodies that arise either in multitransfused patients with hemophilia A or rarely in previously healthy individuals associated with certain acquired diseases.

The wide application of antibodies to immunoassay for F.VIII is impeded by some fundamental and practical barriers: (1) the polyclonality of homologous antibodies directed against more than one functional site or antigenic determinant, (2) The limited availability of high-titer ($>1,000$ Bethesda u/mL) antibodies, (3) the difficulty and expense involved in reproducing the specific radio-labeled antibodies. Those problems may be overcome by the use of monoclonal antibodies possessing well-defined specificity¹⁾. Monoclonal antibodies can be produced in large quantities, and expectedly have a potential to greatly improve the reliability and sensitivity of immunoassay. A number of investigators have reported preparation of monoclonal antibodies to F.VIII²⁻⁹⁾ and application to immunoassay of F.VIII:Ag^{2) 5) 6) 10)}.

In this paper, we report the preparation of a monoclonal antibody (NMC-VIII/1) that shows high-affinity binding to F.VIII:Ag without inhibitory effect on F.VIII:C. A facile

Abbreviations: F.VIII, factor VIII; F.VIII:C, factor VIII coagulant activity; F.III:Ag, factor VIII clotting antigen; vWF, von Willebrand factor; vWF:Ag, von Willebrand factor antigen; RCof, ristocetin cofactor; IRMA, immunoradiometric assay; ELISA, enzyme-linked immunosorbent assay.

two-site solid phase ELISA for F.VIII:Ag using the monoclonal antibody as a second antibody is also described. By this assay we estimated F.VIII:Ag in normal plasma and sera, and hemophilic plasma, and compared them with those estimated by a conventional IRMA using homologous antibodies.

I. MATERIALS AND METHODS

1. Assays for F.VIII and vWF

1) F.III:C was measured by one-stage kaolin-cephalin clotting time assay¹¹⁾ using F.VIII deficient plasma, or amidolytic assay using Coatest® Kit (Kabi)¹²⁾.

2) F.VIII:Ag was measured by two-site solid phase IRMA using homologous antibodies obtained from a patient with severe hemophilia A whose plasma had a high titer of F.VIII inhibitor¹³⁾.

3) VWF:Ag was measured by Laurell's¹⁴⁾ electroimmunoassay using rabbit anti-F.VIII/vWF serum or by ELISA using rabbit anti-vWF serum as a coating antibody and anti-vWF monoclonal antibody (NMC-vW/1 or 4)¹⁵⁾ as a second antibody.

2. Purification of F.VIII

About 1,000 units of a commercial F.VIII concentrate, Koate (Cutter, USA), were applied to 150 ml of Sepharose 4B beads (Pharmacia, Sweden) coupled with 450 mg of IgG of anti-vWF monoclonal antibody (NMC-vW/4). After washing with 3 l of 0.05 M Tris-HCl buffer (pH 7.4) containing 20 mM benzamidinium-HCl, 0.15 M NaCl and 0.2 % NaN₃, the F.VIII was eluted with 0.25 M CaCl₂. The peak value of F.VIII in the eluate fractions was 20 u/ml of F.VIII:C, 30 u/ml of F.VIII:Ag and 0.02 u/ml of vWF:Ag. The peak fraction was further adsorbed by 1/10 (v/v) of NMC-vW/2- or NMC-vW/4-beads at 37°C overnight, and the F.VIII was eluted as described above.

3. Production of monoclonal antibody against F.VIII

BALB/c mice, 6 weeks old, were immunized by intraperitoneal injections of a purified F.VIII fraction (1-2 u F.VIII:Ag in 100 μ l) emulsified with an equal volume of Freund's complete adjuvant. The injection was repeated at 2, 4, 6 and 8 weeks. One week after the final injection, blood was drawn and analyzed for antibody to F.VIII by the inhibitory coagulation assay. The inhibitor titer was approximately 200 Bethesda units/ml. Three days after a booster injection with the F.VIII fraction (4 u F.VIII:Ag in 200 μ l) without adjuvant, the spleen was removed. The spleen cells (1.0×10^8) were fused with X-63 murine myeloma cells (2.0×10^7) in the presence of 50% PEG 4000 (Wako Pure Chemical Industry, Ltd., Japan). The cells were distributed into wells of multiwell microtiter plates and incubated in HAT medium. Antibody-secreting cells were cloned by undergoing limiting dilution two times. The cloned hybrid cells were injected intraperitoneally to BALB/c mice and monoclonal antibody was obtained from ascitic fluid.

4. Detection of F.VIII antibody

1) F.VIII:C inhibitor assay

Equal volumes of material to be tested and normal pooled plasma were mixed and incubated at 37°C for 2 hr. Aliquots were then mixed with plasma from a patient with severe hemophilia A and were tested for kaolin-cephalin clotting time¹⁶⁾.

2) Four-layer IRMA

The culture medium and ascites were assayed by four-layer IRMA according to the method of Fass et al⁴⁾ with a minor modification. Briefly, polystyrene tubes (Shionogi Pharmaco., Japan) were coated with 40 $\mu\text{g}/\text{ml}$ goat anti-mouse IgG (Miles Yeda Co., USA) in bicarbonate buffer (pH 9.6) at 4°C overnight. The tubes were washed twice with 0.05 M barbital buffer (pH 7.9) and by distilled water. Two hundred μl of culture medium or ascitic fluid was incubated in the tubes at 37°C for 2 hr. After washing with barbital buffer, the purified F.VIII fraction (200 μl) was added and incubated at 37°C overnight. After washing, 200 μl of ¹²⁵I-labeled immunoaffinity-purified human anti-F.VIII IgG (3,000–4,000 cpm) was added and incubated at 37°C overnight. Washing buffer contained 0.1% BSA and dilution buffer contained 4% BSA. As negative control, HAT medium, normal mouse serum and two different species of monoclonal antibodies to vWF (NMC-vW/2 and NMC-vW/4) were used in stead of culture medium or ascitic fluid samples. The samples with at least twice the counts of a negative control (HAT medium) were regarded as positive.

5. Fractionation of monoclonal antibody

The ascitic fluid was heated at 56°C for 30 min and centrifuged to remove resultant precipitates. The immunoglobulin fraction was obtained by precipitation at 50% (w/v) ammonium sulfate or by chromatography using Protein A-Sepharose, and was dialyzed extensively against phosphate buffered saline (PBS).

6. Characterization of monoclonal antibody

Immunoglobulin subclass identification was made by Ouchterlony immunodiffusion using specific anti-mouse immunoglobulin antisera (Nordic Immunological Lab, Ltd, and Miles Lab. Ltd., USA). The presence of anti-vWF:Ag and anti-RC of in the monoclonal antibody preparation was assessed by ELISA and RIPA testkit, respectively¹⁷⁾.

7. Affinity-depletion of F.VIII using monoclonal antibody

The IgG (3 mg) of the monoclonal antibody was coupled to 1 ml of CNBr-activated Sepharose 4B beads (Pharmacia). The F.VIII fraction or normal plasma was recyclically applied to the immunosorbent Sepharose 4B column. After the column was washed with 50 ml of 0.05 M Tris-HCl buffer (pH 7.4) containing 20 mM benzamidine-HCl, 0.15 M NaCl and 0.02% NaN₃, F.VIII was eluted with 0.02 M imidazole buffer (pH 6.4) containing 1 M KI and 20% ethylen glycol.

8. NaDodSO₄-polyacrylamide gel electrophoresis

NaDodSO₄-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a discontinuous buffer system¹⁸⁾ using 1-mm thick slab gels consisting of 7.5% polyacrylamide running gel and 3% stacking gel. After electrophoresis (20 mA), gels were fixed in a 0.1% glutaraldehyde solution and subjected to silver staining.

9. ELISA for F.VIII : Ag¹⁹⁾

Immunoaffinity-purified homologous anti-F.III IgG was diluted in 0.05 M bicarbonate buffer (pH 9.6) and a 0.2-ml aliquot was added to wells of microtiter plates (NUNC, Denmark). The plates were incubated at 4°C overnight. After subsequent incubation for blocking with 0.05 M bicarbonate buffer containing 4% BSA at 37°C for 1 hr, the wells were washed with 0.02 M PBS containing 0.05% Tween/20. Serial dilutions of normal pooled plasma, hemophiliac plasma and other samples to be tested were prepared in PBS/Tween 20 containing 4% BSA and added to the wells. Incubation was continued at 37°C

Fig. 1 Isolation of F.VIII by immunosorbent chromatography using NMC-VIII/1 monoclonal antibody.

Immuno-purified F.VIII fraction (F.VIII:C, 11 u; F.VIII:Ag, 50 u) from F.VIII concentrate using anti-vWF monoclonal antibody was applied to an immunosorbent column of Sepharose 4B coupled with the IgG fraction of NMC-VIII/1. After washing the column, the bound protein was eluted with 0.02 M imidazole buffer containing 1 M KI and 20% ethylene glycol. One-ml fractions were collected. F.VIII:C was assayed by an amidolytic method. F.VIII:Ag was assayed by IRMA using homologous anti-F.VIII antibodies.

overnight and the wells were washed again. F.VIII:Ag bound to the coating antibody was detected by sequential incubations with NMC-VIII/1 (ascitic fluids diluted 1 : 1,000 with PBS/Tween 20 containing 4% BSA), peroxidase-conjugated goat anti-mouse F(ab')₂ (TAGO, USA), and o-phenylenediamine as a substrate.

II. Results

The fusion products from one immune mouse spleen were seeded into 240 wells of micro-titer plates. Twelve days later, cells in 6 wells demonstrated a significant production of anti-F.VIII antibody as examined by four-layer IRMA. These were transferred to larger volume (1 ml) culture to allow growth expansion. Finally, the cells in one well were subjected to cloning by limiting dilution twice to establish a stable cell line. The cloned cells were injected intraperitoneally into BALB/c mice pre-treated with Pristan. The antibody in the ascites was designated as NMC-VIII/1 and was studied in detail.

1. Characterization of monoclonal antibody

The immunoglobulin fraction in culture medium of ascites produced by the hybridoma demonstrated the following characteristics. The immunoglobulin was of IgG₁ subclass and appeared as a homogeneous band on SDS-PAGE. A 10⁵ - fold dilution of the ascitic

fluid gave a positive assay by IRMA and the titer of the monoclonal antibody was thus 1×10^5 . No inhibitory activity to F.VIII:C was detected. Anti-vWF:Ag and anti-RCof were absent.

2. Immunosorbent column chromatography using monoclonal antibody (Fig. 1)

The vWF:Ag-free F.VIII fraction, consisting of 11 u of F.VIII:C and 50 u of F.VIII:Ag, was applied to the immunosorbent column (1 ml) 5 times recyclically, and 5 u of F.VIII:C and 33 u of F.VIII:Ag were recovered in the eluate. The ratio of F.FVIII:C/F.III:Ag in the eluate was almost the same as that of the starting F.VIII fraction. In the unadsorbed fraction, neither F.VIII:C nor F.VIII:Ag was detected. When 2 ml of normal plasma was similarly applied to the column, only 0.1 u of F.VIII:C and 0.5 u of F.VIII:Ag were eluted. In this case, 1.1 u of F.VIII:C was detected in the unadsorbed fraction.

3. Immunoabsorption of F.VIII by monoclonal antibody (Fig. 2)

The purified F.VIII protein was visualized on SDS-PAGE as several bands closely located in the range of 80-79 KDa. All these bands were completely diminished by prior adsorption with the NMC-VIII/1 beads but unaffected by treatment with the beads coated with a mixture of anti-fibrinogen, anti-fibrinonectin and anti-IgM IgGs.

4. Competitive binding to F.VIII:Ag of monoclonal antibody (Fig. 3)

To assess whether the monoclonal anti-F.VIII antibody (NMC-VIII/1) competes with human antibodies for binding to F.VIII:Ag, serially diluted solutions of NMC-VIII/1 were incubated with a F.VIII concentrate at 37°C for 2 hr, and then IRMA for F.VIII:Ag was performed using homologous polyclonal antibodies. The monoclonal antibody produced dose-dependent inhibition of F.VIII:Ag binding to human antibodies, but did not attain complete inhibition.

5. ELISA for F.VIII:Ag using monoclonal antibody (Fig. 4)

A standard curve for the measurement of F.VIII:Ag by ELISA was satisfactorily constructed using normal pooled plasma diluted from 1 : 2.5 to 1 : 320. Reading of absorbance

Fig. 2 NaDodSO₄ PAGE of F.VIII after immunoabsorption by the monoclonal antibody.

Two hundred μ l of F.VIII fraction was incubated with 200 μ l of Sepharose 4B beads coupled with a mixture of anti-fibrinogen, anti-fibrinonectin and anti-IgM IgGs or with NMC-VIII/1 IgG. A mixture of approximately 5 mg of each IgG fraction was coupled with one ml of beads. After incubation at room temperature for 2 hr and at 4°C overnight, the supernatant was applied to 7.5% NaDodSO₄ PAGE. The gel was stained by silver staining method after electrophoresis. Lane A is the F.VIII fraction untreated. Lane B is the F.VIII fraction after treatment with a mixture of anti-fibrinogen, anti-fibrinonectin and anti-IgM IgGs and Lane C is the F.VIII fraction after treatment with NMC-VIII/1 IgG.

Fig. 3 Competitive binding to F.VIII:Ag of the monoclonal antibody.

Serially diluted NMC-VIII/1 (each 20 μ l) was pre-incubated with 180 μ l of diluted F.VIII concentrate (approximately 300 u/dl F.VIII:Ag) at 37°C for 2 hr and then IRMA for F.VIII:Ag was performed using 125 I-labeled homologous antibody. ●—● with pre-incubation for 2 hr, ▲—▲ without pre-incubation.

developed by the plasma at greater dilutions than 1 : 640 was unreliable and the lower limit of detection by this method was thus 3×10^{-3} u/ml. We measured F.VIII:Ag of plasma samples obtained from 20 normal subjects, 20 patients with severe hemophilia A, 5 with mild to moderate hemophilia A and of 20 normal sera samples by ELISA using monoclonal antibody (F.VIII:Ag ELISA) and by IRMA using homologous polyclonal antibodies (F.VIII:Ag IRMA). In normal plasma samples, F.VIII:Ag ELISA levels correlated well with those F.VIII:Ag IRMA ($r=0.86$). No F.VIII:Ag ELISA or F.VIII:Ag IRMA was detected in any plasma from the severe hemophiliacs. Considerable amounts of F.VIII:Ag ELISA and F.VIII:Ag IRMA were detected in plasma from 5 mild to moderate hemophiliacs without remarkable difference between the two values. In normal serum samples, the mean value of F.VIII:Ag ELISA was 14 ± 5 u/dl, which was much lower than F.VIII:Ag IRMA (Table 1).

6. Time course study of the change of F.VIII:C and F.VIII:Ag level during clotting (Fig. 5)

We also studied a serial change in the F.VIII:Ag ELISA level during blood clotting. The F.VIII:Ag ELISA level immediately decreased to 20 u/dl within 5 minutes after venipuncture and then declined at a slow rate, whereas the F.VIII:C level assayed by one stage

Fig. 4 Dose-response curve of F.VIII:Ag measured by ELISA using the monoclonal antibody.

Normal pooled plasma (●—●) and plasmas from the patients with mild (□—□), moderate (△—△) and severe hemophilia A (○—○) were diluted with PBS/Tween 20 containing 4% BSA and added to the well coated by immunoaffinity-purified homologous anti-F.VIII IgG. The monoclonal antibody NMC-VIII/1 was used as a second antibody and peroxidase-conjugated goat anti-mouse F(ab')₂ was used as a tracer. Absorbance was measured at 492 nm.

clotting method increased for the first 20 min and then decreased steeply. The F.VIII:Ag ELISA level was 15 u/dl and F.VIII:C was 8 u/dl at 180 min.

III. DISCUSSION

The application of hybridoma technology provides valuable and new information of the structure and function of factor VIII molecule. Since the first report by Muller et al, 8 groups²⁻⁹⁾ have reported preparation of a total of 24 monoclonal antibodies to F.VIII. Seven groups^{2,3,5-9)} produced anti-human F.VIII monoclonal antibodies and the other one group⁴⁾ anti-porcine F.VIII antibody. Five groups^{2,4,6,8,9)} used a purified F.VIII fraction and the others a F.VIII/vWF fraction as the immunogen. Four groups^{2,5,6,10)} applied the antibodies to IRMA. Two groups^{4,8)} used them to study the structure and function of factor VIII protein. Preparation of many other monoclonal antibodies with different specificities to sites of factor VIII molecule is of value currently.

We immunized BALB/c mice with a purified F.VIII fraction and obtained a high titer anti-F.VIII monoclonal antibody by a standard procedure. For the screening for antibody

Table 1 The results of F. VIII:Ag assay by monoclonal ELISA and polyclonal IRMA

Samples	F. VIII:C (u/dl)	F. VIII:Ag (u/dl)	
		Monoclonal ELISA	Polyclonal IRMA
Normal plasma (n=20)	94 ± 24	91 ± 25	97 ± 23
Normal serum (n=20)	11 ± 6	14 ± 5	68 ± 21
Hemophilia A plasma			
Case 1	<1	<0.3	<0.1
2	<1	<0.3	<0.1
3	<1	<0.3	<0.1
4	<1	<0.3	<0.1
5	<1	<0.3	<0.1
6	<1	<0.3	<0.1
7	<1	<0.3	<0.1
8	<1	<0.3	<0.1
9	<1	<0.3	<0.1
10	<1	<0.3	<0.1
11	<1	<0.3	<0.1
12	<1	<0.3	<0.1
13	<1	<0.3	<0.1
14	<1	<0.3	<0.1
15	<1	<0.3	<0.1
16	<1	<0.3	<0.1
17	<1	<0.3	<0.1
18	<1	<0.3	<0.1
19	<1	<0.3	<0.1
20	<1	<0.3	<0.1
* 21	2	75	67
* 22	2	73	64
23	5	11	8
24	5	12	8
* 25	6	35	38

* Case 21, 22 and 25 are patients with typical hemophilia A variants

producing hybridoma, we employed binding assay using ^{125}I -labeled homologous anti-F.VIII antibodies. This assay was confirmed to be specific for anti-F.VIII. The monoclonal antibody we obtained and designated as NMC-VIII/1 showed a high binding affinity for F.VIII with no inhibitory effect on F.VIII:C. Four of 24 monoclonal antibodies reported previously showed no inhibition of F.VIII:C. There have been no reports of an antibody that inhibits F.VIII:C completely.

The adsorption of F.VIII from plasma was relatively weak by the NMC-VIII/1 immunosorbent column but it completely absorbed the F.VIII fraction dissociated from vWF protein. Furthermore, the eluate from the monoclonal anti-F.VIII immunosorbent column had the same ratio of F.VIII:C to F.VIII:Ag as that of the starting material. One explanation for these results is that F.VIII in the F.VIII/vWF complex is not readily accessible to the antibody because the vWF molecule is much larger than the F.VIII molecule. Another possibility is that the antigenic epitope of the F.VIII molecule for the antibody is more exposed by the dissociation from vWF.

There are some advantages in use of the antibody. One is that it can isolate F.VIII without F.VIII:C inactivation involved in other isolation procedures. The second is application of the antibody to immunoassay. There are two ways of using a monoclonal antibody for

Fig. 5 Time course of the change of F.VIII:C and F.VIII:Ag levels during clotting.

Whole blood from a healthy volunteer was subsampled and incubated at 37°C for 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min. At intervals, the clotting was stopped by the addition of 3.8 % sodium citrate. Each sample was centrifuged at 3,000 rpm for 10 min and then F.VIII:C and F.VIII:Ag in the supernatant were assayed.

two-site ELISA, as a coating antibody and a second antibody. NMC-VIII/1 was not suitable for use as a coating antibody in ELISA for F.VIII:Ag, because beads coupled with its IgG adsorbed less than 50% of F.VIII in plasma. Therefore, we established ELISA using polyclonal anti-F.VIII IgG as a coating antibody and NMC-VIII/1 IgG as a second antibody.

We determined F.VIII:Ag in plasma and serum obtained from normal subjects and from patients with hemophilia A. There was no remarkable difference between F.VIII:Ag ELISA level and F.VIII:Ag IRMA. In normal serum, however, the level of F.VIII:Ag ELISA was much lower than F.VIII:Ag IRMA. Immunoassay using the monoclonal antibody obtained by Muller et al.²⁾ did not detect any F.VIII:Ag in serum, but that produced by Brown et al.⁵⁾ detected a higher level of F.VIII:Ag in serum than in normal plasma. In our assay using NMC-VIII/1, the F.VIII:Ag level was decreased in serum but a considerable level still remained. We also examined the change of F.VIII:Ag ELISA level with blood clotting. Despite the initial increase of F.VIII:C, which was considered as F.VIII activation, a reduction of F.VIII:Ag ELISA was seen from the beginning of clotting. These findings indicate that a certain structural change or denaturation occurred in the 80-79 KDa fragment portion of F.VIII molecule early in the process of blood clotting, and that the epitope is

located near or at a site closely related to an early change of F.VIII molecule in blood clotting.

The monoclonal antibody described in this paper is useful not only for establishment of reliable immunoassay such as ELISA but also for the study of the structure and function of F.VIII and F.VIII/vWF.

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●内科疾患のカルシウム代謝とその異常

カルシウムの臨床

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カルシウムは人体のあらゆるところに存在し、あらゆる機能に関与する。したがって、内科疾患のすべてに密接に関連しており、内科学を理解するためにカルシウム代謝とその異常をまず知らなければならない。本書は内科疾患のあらゆる部分にわたってカルシウムの影を探し求め、カルシウムの臨床を通して内科学を再構成しようとしたユニークかつ野心的な試みの産物である。

●内容：総論，第1章 神経系疾患とカルシウム代謝異常，第2章 心血管疾患とカルシウム，第3章 腎疾患とカルシウム，第4章 呼吸器疾患とカルシウム，第5章 消化器疾患とカルシウム，第6章 血液疾患とカルシウム，第7章 内分泌疾患とカルシウム，第8章 リウマチ様関節炎とカルシウム代謝，第9章 膠原病および免疫アレルギー疾患とカルシウム，第10章 物理的原因による疾患とカルシウム，第11章 薬剤とカルシウム代謝異常，第12章 老年病とカルシウム，第13章 悪性腫瘍とカルシウム，第14章 カルシウム代謝検査法，索引