DOWNLOAD PDF FIBRINOGEN AND ITS DERIVATIVES

Chapter 1 : OMIM Entry - * - FIBRINOGEN, B BETA POLYPEPTIDE; FGB

In contrast to the defibrination mediated by the thrombin clotting of fibrinogen, such as in the disseminated intravascular coagulation syndrome, the in vivo induction of defibrination by Ancrod or Reptilase is suprisingly benign and not accompanied by significant changes in most clotting factors or in platelet count.

It is composed of 3 structurally different subunits: Thrombin causes a limited proteolysis of the fibrinogen molecule, during which fibrinopeptides A and B are released from the N-terminal regions of the alpha and beta chains, respectively. The enzyme cleaves arginine-glycine linkages so that glycine is left as the N-terminal amino acid on both chains. Thrombin also activates fibrin-stabilizing factor factor XIII; see and , which in its activated form is a transpeptidase catalyzing the formation of epsilon- gamma-glutamyl -lysine crosslinks in fibrin. Fibrinopeptides, which have been sequenced in many species, may have a physiologic role as vasoconstrictors and may aid in local hemostasis during blood clotting summary by Dayhoff, This result was interpreted as indicating loss of genetic material at the site of the GYPA and GYPB genes presumably related to the de novo translocation. No evidence was found for rearrangement of gamma or beta fibrinogen. By in situ hybridization using probes for GYPA and for FGB, no hybridization was found on the derived chromosome 2, which contained most of 4q In acute and chronic rat models of myocardial ischemia-reperfusion injury, FGB substantially reduced leukocyte infiltration, infarct size, and subsequent scar formation. The year-old blood donor had no symptoms of bleeding tendency or thrombosis. A sister was similarly affected. The mother, who may have had the variant, called Erfurt I, was deceased. In a large study in Copenhagen, Tybjaerg-Hansen et al. The allele frequency was 0. In 3 Italian sibs with dysbetafibrinogenemia with thrombosis, Koopman et al. In 2 families with congenital afibrinogenemia, 1 Italian and 1 Iranian, Duga et al. They reported 2 additional mutations in the FGB gene as the cause of afibrinogenemia The threshold concentrations of epinephrine and ADP required to produce biphasic platelet aggregation and collagen lag time were determined. After accounting for environmental covariates, the adjusted sib correlations for epinephrine, ADP, and collagen lag time were 0. In contrast, adjusted correlations for spouse pairs were The estimated heritabilities were 0. Expression of additional mutants and structural modeling suggested that neither the last 6 residues nor arg is crucial per se for secretion, but prevents protein misfolding by protecting hydrophobic residues in the B-beta C-terminal core. Immunofluorescence and immunoelectron microscopy studies indicated that secretion-impaired mutants were retained in a pre-Golgi compartment. In addition, expression of FGB, FGG, and angiopoietin-2 ANGPT2; chimeric molecules demonstrated that the B-beta C-terminal domain prevented the secretion of single chains and complexes, whereas the gamma C-terminal domain allowed their secretion. There were several fibrinogen locus SNPs associated with lower fibrinogen that were exclusive to African Americans.

Chapter 2 : Latex Immunoturbidimetric Assay for Soluble Fibrin Complex | Clinical Chemistry

When factor VIII was added together with both fibrinogen and its derivatives, the amount of thrombin generated was even greater, about % larger than the amount which was generated in the presence of equal concentrations of only intact fibrinogen plus factor VIII.

The anti-D-dimer monoclonal antibody of the present invention may be effectively used as a diagnotic agent for screening and detecting in-vivo D-dimer, and high molecular weight cross-linked fibrin and its derivatives containing the D-dimer since the monoclonal antibody specifically reacts with D-dimer, and cross-linked fibrin and its derivatives containing the D-dimer, which do not bind to human fibrinogen or fibrin. TECHNICAL FIELD The present invention relates to a monoclonal antibody against a high-molecular cross-linked fibrin including D-dimer and its corresponding epitope produced in a mouse, and a diagnosis agent for detecting D-dimer and cross-linked fibrin or its derivatives containing D-dimer by using the antibody. More particularly, the present invention relates to a monoclonal antibody capable of being effectively used as a diagnosis agent for quantifying D-dimer and cross-linked fibrin or its derivatives containing D-dimer since the monoclonal antibody specifically reacts to human D-dimer and is manufactured with a high activity, wherein the monoclonal antibody is derived from a mouse and specifically binds to an amino acid sequence composed of amino acids to from an N terminus of a D domain beta-chain, or amino acids to from the N terminus of the D domain beta-chain and amino acids to of an alpha-chain, and a diagnosis agent for detecting D-dimer and cross-linked fibrin or its derivatives containing D-dimer by using the antibody. The former is a mechanism for forming a thrombus and the latter is a mechanism for dissolving the thrombus. A fibrin is a major component constituting a thrombus and digests into several fibrin degradation products FDP through fibrinolysis. The formation and dissolution of fibrin substantially occur at the same times, and D-dimer is an important marker among the FDP produced in fibrin dissolution process. D-dimer is a final degradation product produced when an insoluble fibrin, in which gamma chains are cross-linked to each other by a factor XIII, is degraded by plasmin. It was known that FDP and D-dimer are detected at a higher concentration in plasma of patients suffering from various diseases such as pulmonary embolism, deep vein thrombosis, tumor surgery, disseminated intravascular coagulation, myocardial infarction, trauma, cancer, kidney and liver function impairment than in healthy humans. In particular, FDP and D-dimer have been the most used markers for diagnosing pulmonary embolism and deep vein thrombosis. Because pulmonary embolism and deep vein thrombosis do not have any distinct symptoms showing that the patients may develop pulmonary embolism and deep vein thrombosis, which eventually may lead to death. Also, test results are significantly different among the diagnosis agents since the different monoclonal antibodies; which adopted in each of the said diagnosis agents recognize different cross-linked fibrin degradation products, for instance, preferential binding of low molecular weight fibrin degradation product or of high molecular fibrin products. In particular, an erroneous diagnosis may be made in some test kits using a diagnostic agent specific to low-molecular weight fibrin degradation products since fibrin derivatives in plasma are present as a partially degraded form of cross-linked fibrin rather than a fully digested D-dimer form, especially in the case of the patients suffering from disseminated intravascular coagulation DIC syndrome for a long time and being subject to an anti-coagulation treatment see Abraham Konberg, Blood , vol 80, No 3, An ELISA diagnostic method using the monoclonal antibody produced in the present invention had excellent quantitative results than other diagnostic reagents in the test of plasma. In the present invention, D-dimer-specific monoclonal antibodies were produced from hybridoma cell and purified from the cell culture supernatant, and then applied to the quantification of D-dimer or cross-linked fibrin or its derivatives containing D-dimer in the human body fluids through method of ELISA, LIA, etc. Accordingly, the present invention is designed to solve the problems of the prior art, and therefore it is an object of the present invention to provide a murine monoclonal antibody capable of being effectively used as a diagnosis agent for screening and detecting D-dimer and cross-linked fibrin or its derivatives containing D-dimer since the monoclonal antibodies produced are highly reactive to D-dimer. In order to accomplish the above object, the present invention provides a monoclonal

antibody that specifically reacts to D-dimer and cross-linked fibrin or its derivatives containing D-dimer, wherein D-dimer is produced by converting human fibrinogen into fibrin using an enzyme thrombin, followed by digesting with plasmin. The monoclonal antibody of the present invention recognizes D-dimer and cross-linked fibrin or its derivatives containing D-dimer at the same time. D-dimer is produced by completely degrading cross-linked fibrin by plasmin. When an antibody reacts with only low-molecular weight fibrin degradation product, the high molecular weight fibrin degradation product may not be recognized by same antibody. Therefore, the concentration in the samples in which cross-linked fibrin is not degraded or partially degraded may be measured as much lower than their actual level. Thus, it is the most preferred to measure D-dimer level in plasma that the monoclonal antibodies used in D-dimer test recognize the cross-linked fibrin degradation products as well as D-dimer, but does not react with fibrinogen or its derivatives. The antibody of the present invention preferably reacts to a site including an N-terminal amino acid sequence to of a beta-chain set forth in SEQ ID NO: Also, the present invention provides for diagnostic agents for detecting D-dimer and cross-linked fibrin products, including: According to the present invention, the marker is preferably selected from the group consisting of horseradish peroxidase HRP alkaline phosphatase. The substrate preferably includes at least one coupler selected from the group consisting of o-phenylenediamine and hydrogen peroxide. Lanes 1, 2 and 3 represent the patient plasmas exhibiting the different results in the ELISA method, respectively, and Lane 4 represents the column eluents of the patient plasmas exhibiting the same results. The present invention provides a fusion cell line that secrets anti-D-dimer monoclonal antibody, the fusion cell line being obtained by producing D-dimer from human fibrinogen, purifying D-dimer and immunizing a mouse with D-dimer. The fusion cell line producing the monoclonal antibody was prepared by immunizing mouse with D-dimer protein and fusing spleen cells from the immunized mouse with a mouse myeloma cell line. To select a positive clone of the fusion cell line producing the monoclonal antibody of the present invention, an enzyme-linked immunoassay ELISA was carried out on the purified D-dimer coated plate for screening, and a limiting dilution was carried out to separate a monoclonal antibody from the selected positive clone of high sensitivity. Finally, the monoclonal antibodies proven to have an anti-D-dimer specificity were named B2, C3, B4 and C5, respectively. The monoclonal antibodies of the present invention produced from the fusion cell line are IgG1-type kappa light chains and have a high specificity to a human D-dimer. Also, the monoclonal antibodies do not exhibit any cross-reactivity to structurally similar fibrinogen and its derivatives. It was found that an antigen recognition region of the monoclonal antibody B4, which is determined by western-blotting to the fragments of the purified D-dimer protein degraded by trypsin, chymotrypsin and CNBr, is composed of sites including N-terminal amino acid sequence to of a beta chain and N-terminal amino acid sequence to of the alpha chain. It was found from the experimental fact that the protein including the N-terminal amino acid sequence to of a beta chain is recognized by the monoclonal antibody B4, while the protein including the N-terminal amino acid sequence to of the beta-chain is not recognized by the monoclonal antibody B4. Also, a protein encoded by the N-terminal amino acid sequence to of the alpha chain is weakly recognized by the monoclonal antibody B4 in comparison with the total D-dimer protein because the alpha chain and the beta chain are separated far from each other. Therefore, it was confirmed that the monoclonal antibody B4 recognizes a certain region of the unique string structure consist of twisting alpha, beta and gamma chain see Brown J. Also, the present invention provides a diagnostic agent for screening and detecting D-dimer in plasma and cross-linked fibrin or its derivatives containing D-dimer, using the anti-D-dimer monoclonal antibody produced as described above. The diagnostic agents of the present invention are composed of the anti-D-dimer monoclonal antibody as a primary antibody; a D-dimer monoclonal antibody produced as a secondary antibody by conjugating a marker such as horseradish peroxidase with the said antibody; and a substrate solution including a material for inducing color development by reacting with the marker. D-dimer monoclonal antibody used as a secondary antibody conjugate has a marker conjugated thereto. At this time, horseradish peroxidase HRP , alkaline phosphatase, and other suitable markers may be used as markers. The presence of the D-dimer antigen may be determined by the binding of the antigen present in the sample to a primary antibody immobilized on solid surface. Secondary antibody conjugate then forms a sandwich complex. The substrate solution for inducing color development by reacting with the marker includes a buffer

and a coupler such as o-phenylenediamine, hydrogen peroxide solution, etc. The substrate solution may develop colors by reaction of the marker conjugated to the monoclonal antibody, and therefore the presence and an amount of the antigen may be determined by measuring a level of the color development. Ultimately, the concentration of D-dimer or high molecular weight cross-linked fibrin degradation product in tissue sections, whole blood, plasma, cells or like may be quantitatively analyzed with the diagnosis agents on the crude samples themselves or by using ELISA, western blotting, immunoprecipitation or like. Hereinafter, preferred embodiments of the present invention will be described in detail. However, it should be understood that the description proposed herein is just a preferable example for the purpose of illustrations only, not intended to limit the scope of the invention. Example 1 Preparation and Purification of Human D-Dimer Protein As an antigen D-dimer was obtained by converting human fibrinogen into fibrin using an enzyme thrombin, cutting fibrin with an enzyme plasmin and purifying the resultant fragments of fibrin. That is to say, a reaction buffer was prepared by dissolving 50 mM Tris-HCl p Fibrin mass was thoroughly washed with distilled water, frozen with liquid nitrogen, milled, and then put into a reaction buffer. After hour reaction, unit of aprotinin was added to stop the reaction. Gel permeation chromatography was used to purify D-dimer from the produced fibrin degradation products. At this time, the buffer used is 50 mM phosphate solution pH7. After the colonies were then formed, an enzyme-linked immunoassay ELISA was carried out using the purified D-dimer to select positive colonies, but to exclude the positive colonies against fibrinogen and its derivatives. In particular, the purified D-dimer and fibrinogen and its derivatives, respectively, were thoroughly mixed in a coating buffer 0. Then, an excessive amount of the non-reacted antibody was removed off, goat-derived anti-mouse IgG antibody KPL to which horseradish peroxidase HRP binds, o-phenylenediamine Sigma which is a substrate of HRP, and hydrogen peroxide solution H2O2 was sequentially added to confirm whether or not the specifically bound antibody is present in the culture solution. As a result, the hybridoma clones, which exhibit the reactivity to D-dimer protein but do not react to fibrinogen and its derivatives, were selected as positive clones that secret the antibody against D-dimer. A limiting dilution was carried out to separate a monoclonal clone from the positive clones selected in the above-mentioned procedure. For this purpose, all of the cell lines present in the well plate were taken and suspended to count the cells. Then, the cells were dividedly added at an amount of 0. At this time, the clones, grown in the plate including the smallest amount of the cells, were selected and grown again, and the above-mentioned procedure was repeated, if necessary. Finally, the clone grown in the most diluted density of the plate was considered to be a monoclonal clone. By carrying out the procedure, the monoclonal antibodies, finally proven to have an anti-D-dimer specificity, were named B2, C3, B4 and C5, respectively. Example 3 Characterization of Prepared Monoclonal Antibodies B In order to determine immunoglobulin antibody types of the monoclonal antibodies, a kit Pierce for determining mouse immunoglobulin type was used. Firstly, wells of ELISA plate were coated with an antigen, the purified monoclonal antibodies were added thereto, and rabbit-derived anti-mouse immunoglobulin antibodies, respectively, specific to the mouse immunoglobulin types were added, and then goat-derived anti-rabbit immunoglobulin antibodies to which horseradish peroxidase HRP binds were added to screen whether or not positive results are found in the monoclonal antibodies. As a result, it was confirmed that all the monoclonal antibodies are kappa light chains of IgG1, as shown in FIG.

Chapter 3 : ANTIGENICITY OF HUMAN FIBRINOGEN AND ITS DERIVATIVES

The measurement of fibrinogen and its derivatives. II. Determination of total immunoreactive fibrinogen in the presence of soluble fibrin monomer complexes (cryoprofibrin complexes).

Most heart attacks and strokes are caused by a blood clot that obstructs the flow of blood to a portion of the heart or the brain. No blood flow, and thus no oxygen, means no life to the heart or brain cells. Blood clots kill more than , Americans every year. Low-dose aspirin and certain nutrients provide partial protection against abnormal blood clots, but a newly identified clotting factor, fibrinogen, mandates that additional measures be taken to prevent heart attack and stoke. High levels of fibrinogen predispose a person to coronary and cerebral artery disease, even when other known risk factors such as cholesterol are low. Fibrinogen elevation in cigarette smokers, for example, has been identified as a primary mechanism causing heart disease and stroke. Cigarette smoking increases cardiovascular disease risk and it raises fibrinogen levels in the blood. Published studies documenting the dangers of cigarette smoking show that cigarette smokers who suffer from cardiovascular disease also have high fibrinogen levels. In fact, high fibrinogen levels may be a more powerful predictor of cardiovascular mortality than cigarette smoking itself. The role of fibrinogen in the development of cardiovascular disease has been fully confirmed by the results of all relevant studies conducted during the past 10 years. High fibrinogen levels have at least as great a predictive value as any other known risk factor, such as elevated LDL cholesterol, elevated triglycerides, obesity and diabetes. In persons with a family history of heart disease, fibrinogen levels are high. Fibrinogen levels are primarily genetically inherited, meaning that fibrinogen may be the genetic factor causing familial premature heart disease. Also, exposure to cold increases fibrinogen levels by 23 percent, and mortality from heart attack and stroke are higher in winter than in summer. Fibrinogen hinders blood flow and oxygen delivery by deforming red blood cells, causing red-cell aggregation and a thickening of the blood, all of which lead to diminished circulation. Fibrinogen binds blood platelets together, thus initiating abnormal arterial blood clots. Fibrinogen is then converted to fibrin, which is the final step in the blood-clotting cascade. Fibrinogen contributes to the development of atherosclerosis by incorporating itself into the arterial plaque. Further, fibrinogen and LDL cholesterol work together to generate atherosclerotic plaques. Fibrinogen initiates the atherosclerotic plaque, then converts to fibrin and serves as a scaffold for LDL cholesterol in the atherosclerotic plaque that slowly occludes an artery. Fibrinogen and its derivatives trigger a variety of other mechanisms thought to be involved in the atherosclerotic process. Fibrinogen and LDL cholesterol have a synergistic effect in promoting atherosclerosis, though fibrinogen may play a more important role in the development of atherosclerotic lesions. Most heart attacks occur because a blood clot forms inside a coronary artery and chokes off the blood supply to the heart. Most strokes, on the other hand, occur because a blood clot forms inside a cerebral artery and blocks the blood supply to the brain. In either case, it is crucial to take steps to reduce the risk of fibrinogen causing an abnormal arterial clot. Platelet-aggregation inhibitors reduce the risk that fibrinogen will cause an abnormal blood clot. Platelet-aggregation inhibitors include aspirin, green tea , ginkgo biloba and vitamin E. For optimal protection against the formation of arterial blood clots, it makes sense to utilize therapies that support lowering elevated fibrinogen levels. In addition, high vitamin A and beta-carotene serum blood levels have been associated with reduced fibrinogen levels in humans. For example, animals fed a vitamin A deficient diet have an impaired ability to break down fibrinogen, but when they are injected with Vitamin A, they produce tissue plasminogen activator that breaks down fibrinogen. In addition, omega 3 fatty acids have been shown to support the lowering of fibrinogen in women with elevated fibrinogen levels. The daily amount of omega 3 fatty acids required to produce a fibrinogen-lowering effect is 6 grams. Elevated homocysteine levels have been shown to block the natural breakdown of fibrinogen by inhibiting the production of tissue plasminogen activator. Folic acid and vitamin-B6 included in Life Extension Mix -- LEM show strong implications that may help reduce elevated homocysteine levels. One of the more interesting studies involved the use of vitamin C to break down excess fibrinogen. In a report published in the journal Atherosclerosis, heart-disease patients were given either 1, or 2, mg a day of vitamin C to assess its effect on the breakdown of fibrinogen. At 1, mg a day, there was no

detectable change in fibrinolytic activity fibrinogen breakdown or cholesterol. At 2, mg a day of vitamin C, however, there was a percent decrease in the platelet-aggregation index, a percent reduction in total cholesterol, and a percent increase in fibrinolytic activity. For maximum fibrinogen-lowering support, the proteolytic enzyme bromelain may be the most effective nutrient supplement. For those seeking to lower elevated fibrinogen levels, two to six capsules a day of standardized bromelain should be considered. Some non-pharmacologic ways of lowering fibrinogen include stopping smoking, avoiding obesity, lowering LDL cholesterol and avoiding exposure to cold. In patients whose fibrinogen levels were over , Bezafibrate lowered fibrinogen levels by 45 percent. Bezafibrate has been used extensively in Europe since to lower LDL cholesterol by 20 to 30 percent, and increase beneficial HDL cholesterol. It has more than 9 million patient-years of safety documentation.

Chapter 4 : Fibrinogen (Blood Clots) Lowering Support -- The Enzyme Bromelain

The method proved to be superior to all known isolation methods in respect to ease of use and yield, since fibrinogen could be isolated in one step out of plasma with a recovery of more than 95% when compared to the immunologically measurable amounts of fibrinogen.

Soluble fibrin complex SFC , composed of fibrin monomer and fibrinogen derivatives, is known to exist in the circulating blood in patients with thrombosis. Its detection and quantification are useful for obtaining information about the condition and degree of intravascular coagulation in early-stage thrombosis, but there is no rapid method to measure SFC in plasma for clinical use. We obtained a monoclonal antibody that specifically reacts with SFC, with desAA-fibrin as the immunogen, and developed a rapid and sensitive latex immunoturbidimetric assay LIA using latex-immobilized anti-SFC monoclonal antibody. The assay system was based on the increase in turbidity induced by the reaction of the latex-immobilized anti-SFC monoclonal antibody with SFC in plasma, and the assay procedure was fully automated on a Hitachi analyzer. SFC concentrations in plasma from patients with thrombotic diseases [mean SD , In terms of linearity, precision, and sensitivity, the LIA, performed on a Hitachi automated analyzer, may be useful for measurement of SFC in plasma. Fibrin monomer FM 1 and its derivatives in blood, produced by the cleavage of one or both A peptides and both A and B peptides from fibrinogen by thrombin, are found in early-stage thrombosis 1. When produced in blood, they form complexes with fibrinogen and exist as soluble complexes called soluble fibrin complex SFC 2 3. Because increased SFC concentrations in plasma indicate that thrombin has converted fibrinogen to fibrin, increased SFC is considered to be a molecular marker of imminent thrombotic events 4 5 6 7. Other hemostatic molecular markers, such as D-dimer and thrombin-antithrombin TAT complexes, have been developed for the diagnosis of disseminated intravascular coagulation DIC. These markers are very sensitive but not specific for the diagnosis of DIC. D-Dimer reflects the combined effect of both coagulation and fibrinolysis. Similarly, TAT complexes may reflect thrombin generation but not the actual thrombin activity in vivo. Later, Dempfle et al. However, NaSCN pretreatment, used to expose the SFC sites recognized by the antibody, also exposed sites of fibrin degradation products, such as cross-linked fibrin degradation products To solve these problems, we successfully obtained a MoAb that specifically reacts with FM, with desAA-fibrin as the immunogen, in the presence of an antipolymerant peptide Gly-Pro-Arg-Pro Here we report a rapid and sensitive latex immunoturbidimetric assay LIA that uses latex-immobilized SFC-specific antibody and performed on a Hitachi automated analyzer. All chemicals and reagents were of the highest available grade. The Japanese scoring system takes into account underlying disease, bleeding symptoms, organ failure, and global coagulation tests fibrinogen, fibrin degradation products, platelet count, and prothrombin time. Patients with DIC were as follows: Blood samples were collected in plastic tubes containing 0. This was established previously in our laboratory Briefly, the reactivity of F to fibrinogen, fibrin, and their derivatives was as follows: The protein concentration in the IgG fraction was estimated based on the absorbance at nm. After 1 h, the latex-immobilized antibody was washed twice by centrifugation and then suspended in 0. After the addition of hirudin at a final concentration of 2. The pellet was solubilized by treatment with 0. The concentration of solubilized FM was determined according to the following equation: The polypeptide chain composition of fibrinogen, fibrin, and their derivatives was analyzed by sodium dodecyl sulfateâ€”polyacrylamide gel electrophoresis. Stock working calibrators were freeze-dried and prepared by diluting the stock with distilled water before use. After another 5 min, the SFC concentration was calculated from the difference in absorbance between the two signals the absorbance at 5 and 10 min at two wavelengths primary wavelength, nm; secondary wavelength, nm. A nonlinear six-point calibration curve prepared with the SFC calibrators was used to calculate the values of the plasma samples. Regression analysis was used to investigate the effect of sample treatment with and without NaSCN in our assay system. Results and Discussion reactivity of anti-sfc antibody f We examined the effect of sample treatment with and without NaSCN in our assay system, using 50 samples from patients with DIC. Regression analysis of the data gave the following equation: This indicated that the LIA is suitable for the Hitachi automated analyzer. We

therefore used F to prepare a latex reagent. The following data were obtained with the latex-immobilized F reagent reagent 2. We measured a series of working calibrators, using assay conditions for the Hitachi The absorbance produced by the latex agglutination reaction increased with decreasing pH of reagent 1, but fibrinogen in plasma precipitated at pH values below 6.

Chapter 5 : Fibrinogen and its derivatives in relationship to ancrod and reptilase â€" Northwestern Scholars

The binding between complementary polymerization sites of fibrin monomers plays an essential role in the formation of the fibrin clot. One set of polymerization sites involved in the interaction.

Chapter 6 : Fibrinogen and its derivatives: cofactors in the intrinsic generation of thrombin.

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