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USAGE OF MONOCLONAL ANTIBODIES FOR DETERMINATION OF LOCALIZATION OF ANTIGENIC DETERMINANTS AND FIBRIN POLYMERIZATION SITES WITHIN FIBRINOGEN AND FIBRIN MOLECULES AND THEIR APPLICATION IN TEST-SYSTEMS FOR DIAGNOSTICS AND THE THREAT OF THROMBUS FORMATION

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It was shown by monoclonal antibodies that BβN-region of fibrin desA molecule (Bβ1-53) comprises the polymerization site including the peptide bond Bβ14-15. This site participates in the second stage of fibrin polymerization — lateral association of protofibrils. In the Bβ15-53 fragment was also found the site called «C», which together with the site «A» participate in the first stage of polymerization — the protofibrils formation. The model of the primary intermolecular interaction of fibrin was designed. It was found by monoclonal antibodies II-4d the site («c») in the N-terminal half of γ chain of the fibrin D-region. This site participates in the protofibrils formation and is complement to site «C» as we assume.

We have discovered two neoantigenic determinants. One of these determinants exposes within the coiled-coil fragment Bβ126-135 of fibrin as a result of fibrinopeptide A splitting off from fibrinogen by thrombin. The structural rearrangements discovered in this site of the fibrin molecule are necessary for the following protofibrils lateral association. The second neoantigenic determinant is localized in the fragment Bβ134-190 of D-dimer formed after plasmin degradation of fibrin stabilized by FXIIIa. We have obtained the fibrin-specific monoclonal antibodie FnI-3C to the first determinant and D-dimer-specific mAb III-3b to the second one.

Three monoclonal antibodies were obtained against the αC-region of fibrin(ogen) molecule. It has been experimentally shown by of one of them that αC-domains is connected with the fibrinopeptides B in fibrinogen and fibrin desA molecules, but removes from the core of the molecules after fibrinopeptides B splitting off by thrombin. Two other monoclonal antibodies specifically inhibit the fibrin polymerization by blocking two unknown polymerization sites within the αC-region.

The test-systems for the soluble fibrin and D-dimer quantification in human blood plasma were designed on the basis of monoclonal antibodies FnI-3C and III-3b as «catch»-antibodies and one II-4d as a «tag»-antibody, respectively. The clinical trials of the test-systems were carried out in Ukraine. It was shown that for the prediction of postoperative thrombotic complications and monitoring the efficiency of antithrombotic therapy the simultaneous quantification of soluble fibrin and D-dimer before the operation and at different time intervals after the operation is required. Only in this case it is possible to get information about the state of the balance between blood coagulation and fibrinolytic systems, and determine the degree of the threat of thrombosis.

Key words: fibrinogen, fibrin, monoclonal antibodies, thrombosis, diagnostic test systems.

Fibrinogen (Fg) is a protein playing the main role in functioning of blood coagulation system [1]. It has a dimeric structure where two subunits are formed by three polypeptide chains: Aα, Bβ and γ. The fibrinogen molecule consists of a central E, two peripheral D-regions and two extended αC-regions.

The E-region, consisting of (Aα1-104, Bβ1-133, γ1-72)$_2$, is formed by the N-terminal segments of all six polypeptide chains. The two D-regions, each comprising of Aα105-219, Bβ134-461 and γ73-411 in the C part of the molecule, have the globular βC232-461 and γC170-411 nodules. The extended αC-regions

(Aα220-610) consist of an unstructured flexible αC-connector (Aα220-391) and a more structured αC-domain (Aα392-610) [2, 3].

The peripheral D-regions are connected with the central E-region by two long flexible coiled-coil connectors each of which consists of polypeptide fragments Aα48-161, Bβ79-193, γ23-135. The N-parts of these fragments include Aα48-104, Bβ79-133, γ23-62 and belong to the E-region. The C-parts Aα105-161, Bβ134-193, γ63-135 belong to the D-region (Fig. 1).

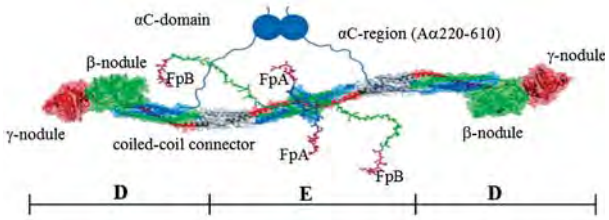

Fig. 1. **The model of fibrinogen molecule created with graphics system PyMol on the base of X-ray and NMR analysis of fibrin(ogen) fragments [2].** Aα-chains — blue; Bβ-chains — green; γ-chains — red

The splitting off of fibrinopeptides A (FpA) — Aα1-16 from fibrinogen (Fg) by thrombin results in the formation of desA fibrin (Fn) with two exposed polymerization sites called A-knobs [4]. The two A-knobs interact with a-holes in the two D-regions of the other Fn molecules forming protofibrils. The protofibrils associate laterally, giving rise to fibrils and, finally, the three-dimensional fibrin net. At the stage of protofibril and fibril formation thrombin splits off of fibrinopeptides B (FpB) — Bβ1-14 from Fn desA molecules exposing the B-knobs which are involved in the process of protofibril lateral association interacting with the b-holes in the D-regions of the other molecules [5].

Two steps of fibrin polymerization — protofibrils formation and their lateral association — are carried out by the intermolecular and interprotofibril binding of specific polymerization sites [6, 7]. Polymeric fibrin is stabilized by factor XIIIa [8]. After polymeric fibrin formation plasminogen is activated on fibrin surface by tPA and transformed into plasmin [9, 10]. Plasmin splits polymeric fibrin stabilized by factor XIIIa forming E-fragment and D-dimer.

Monoclonal antibody (mAb) can be obtained against antigenic determinant (epitope), which can coincide or be located closely to the polymerization site of fibrin molecule. Such a mAb presumably inhibits fibrin polymerization by blocking the polymerization site or the neighboring one. MAb can also «recognize» minor alterations in the epitope conformation. That is why mAbs have been used as molecular probes for localization of unknown antigenic determinants and/or polymerization sites [11, 12].

The tactics of our investigations was following:

- obtaining of antigen for mice immunization: native or slightly denaturated fibrin(ogen) molecules or their fragments;
- obtaining of mAbs;
- selection of mAbs and obtaining their Fab-fragments, which both specifically inhibit fibrin polymerization;
- electron microscopy for determination of fibrin polymerization stage, which is inhibited by the mAb and Fab-fragment.
- epitope localization in fibrin(ogen) molecule for mAb — inhibitor;
- synthesis of the peptide, which imitates the amino acid sequence of fibrin molecule in the monAb epitope region;
- testing of the synthetic peptide inhibitory action on fibrin polymerization.

Three types of monoclonal antibodies of different specificity have been obtained against the N-terminal disulphide knots of fibrinogen and fibrin. Their effects on distinct stages of fibrin polymerization have been studied. These antibodies were shown to be directed against various epitopes of the Bβl-53 fragment of the fibrinogen molecule. The mAbs had different effects both on the rate of protofibril lateral aggregation and on the final turbidity of fibrin clots. The mAbs were of three specificities: mAb 2d-2a and its Fab-fragment inhibited the rate of lateral aggregation of protofibrils and decreased the turbidity of the final clot; those from clone B-4C accelerated the polymerization, but did not affect the clot turbidity; and those from clone D-IB did not have any effect on either fibrin polymerization or final clot turbidity and served as the negative control. The most interesting results were obtained with mAb 2d-2a, epitope for which was localized in fibrin desA fragment including the peptide bond Bβ14-15 [13]. This mAb and its Fab-fragment inhibited the lateral association of fibrin desA protofibrils (Fig. 2) by blocking the site comprising amino acid residues located around the peptide bond Bβ14-15. We suggested that N-terminal part of fibrin desA Bβ-chain concludes one of the active sites involved in protofibril lateral

association. Fibrinopeptide B does not need to be split off for functioning of this site. Interestingly that only one mAb molecule can bind to one of two its epitopes in dimeric fibrin desA molecule blocking one peptide bond Bβ14-15 and inhibiting 60% of fibrin polymerization. In such a case, thrombin may split off only one fibrinopeptide B from fibrinogen. However, two Fab-fragments of this mAb can bind to both epitopes in fibrin desA, blocking them and inhibiting about 100% of polymerization. This mAb has been used as catch-antibody in our ELISA test-system for fibrinogen quantification in human blood plasma after its dilution in 200 times [14].

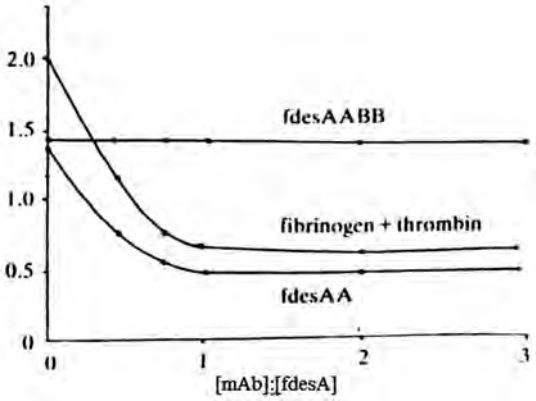

Fig. 2. **Dependence of the rate of protofibrils lateral association (V) on the concentration of mAb 2d-2a**

D-dimer of human fibrin was also used as antigen to obtain monoclonal antibodies. We have obtained 16 hybridomas producing mAbs of different specificity. MAb III-3b binds D-dimer with $K_d = 1.4 \cdot 10^{-10}$ M without cross-reaction with fibrinogen and fibrin. The epitope for this mAb is located in fibrin(ogen) fragment Bβ134–190 (Fig. 3). The latter site is buried in the coiled-coil structure of fibrin(ogen), but it is exposed as a neoantigenic determinant (NAD) in D-dimer upon plasmin hydrolysis of fibrin [15]. MAb III-3b has been used in our ELISA immunodiagnostic test-system as a catch-antibody for quantification of D-dimer in human blood plasma [16].

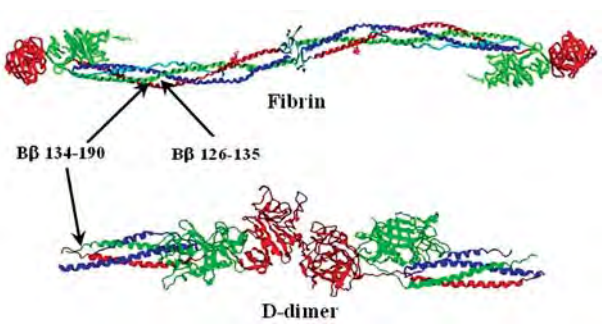

Fig. 3. **The scheme of epitopes localization for mAb I-3c and mAb III-3b in fibrin and D-dimer molecules, respectively**

Only two of these 16 hybridomas produced mAbs of the IgG-class that inhibited fibrin polymerization. MAb II-4d inhibited fibrin polymerization to 100% at a molar ratio mAb:fibrin = 1.0. Fab-fragments of the mAb inhibited fibrin polymerization completely at the same molar ratio (Fig. 4, *1*). The epitopes for the mAbs studied are situated in the NH_2-terminal part of the γ-chain in fibrin D-domain. Electron microscopy showed that fibrin was in monomeric form in the presence of these mAbs or their Fab-fragments (Fig. 4, *2*). Thus, these mAbs inhibit the initial step of fibrin polymerization, i.e. protofibril formation. Only one site of protofibril formation located in COOH-terminal half of the D-domain γ-chain is known now named «a» site, which is complementary to the «A» site in the central E-domain of fibrin molecule. Our experiment with immobilized GPRP showed that the «a» site in fibrin D-fragment preserved its binding activity to GPRP when the D-fragment was complexed with mAbs-inhibitors of fibrin polymerization. Thus, these two mAbs inhibit fibrin polymerization not by blocking the sites «a», but either by blocking another (inconsistent with «a») unknown specific site of polymerization in D-domain or by steric hindrance of highly organized fibrin polymerization process [17]. MAb II-4d has been used in three our immunodiagnostic test-systems for fibrinogen, soluble fibrin and D-dimer quantification in human blood plasma as the tag-antibody [14, 16, 18]

Four mAbs of the IgG_1 class to the thrombin-treated N-terminal disulfide knot of fibrin, secreted by various hybridomas, have been selected. Epitopes for two mAbs I-3C and

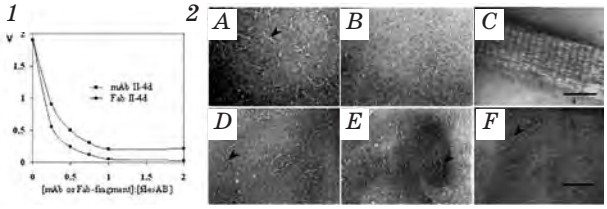

Fig. 4. 1 **— Dependence of the velocity of fibrin polymerization (V) on the molecular ratio of mAb II-4d and it Fab-fragments to fibrin desAB. *2* — Electron microscope images of fibrin desAB polymerizing in the presence (*A, B, C*) and in the absence (*D, E, F*) of mAb II-4d after 3, 5 and 7 min, correspondingly. There were no difference between electron microscope images obtained in the presence of Fab-fragments and mAb II-4d**

III-10d were located in human fibrin fragment Bβ15–26, and epitopes for mAbs I-5G and I-3B were in fragment Bβ26–36. Three of these mAbs, I-5G, I-3B and III-10D as well as their Fab-fragments decreased the maximum rate of fibrin desA and desAB polymerization up to 90–95% at a molar ratio of mAb (or Fab-fragment) to fibrin of 1 or 2. The fourth mAb I-3C did not influence on fibrin desAB polymerization and inhibited by 50% the maximum rate of fibrin desA polymerization. These results suggest that these mAb-inhibitors block a longitudinal fibrin polymerization site. As the mAbs retard both fibrin desAB and fibrin desA polymerization, one can conclude that the polymerization site does not coincide with polymerization site 'B' (Bβ15-17). To verify this suggestion, the polymerization inhibitory activity of synthetic peptides BβSARGHRPLDKKREEA(12–26), BβLDKKREEA(19–26), BβAPSLRPAPPPI(26–36), BβAPSLRPAPPPISGGGYRARPA(26–46) and BβGYRARPA(40–46), which imitate the various sequences in the N-terminal region of the fibrin Bβ-chain, have been investigated. Peptides Bβ12–26 and Bβ26–46, but not Bβ40–46, Bβ19–26, and Bβ26–36, proved to be specific inhibitors of fibrin polymerization. The IC_{50} values for Bβ12–26 and Bβ26–46 were $2.03 \cdot 10^{-4}$ and $2.19 \cdot 10^{-4}$ M, respectively. Turbidity and electron microscopy data showed that peptides Bβ12–26 and Bβ26–46 inhibited the fibrin protofibril formation stage of fibrin polymerization. The conclusion was drawn that fibrin fragment Bβ12–46, named as the site «C», took part in fibrin protofibril formation simultaneously with site «A» (Aα17–19) prior to removal of fibrinopeptide B. A model of the intermolecular connection between fragment Bβ12–46 of one fibrin desA molecule and the D-domain of another has been constructed (Fig. 5) [7].

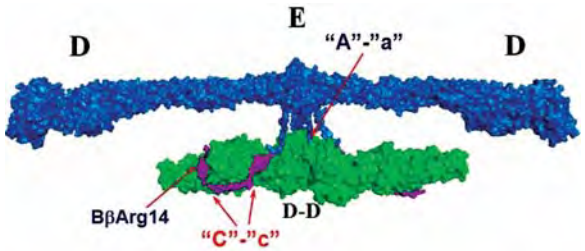

Fig. 5. **The model of the intermolecular connection between the D-domain of one fibrin desAA molecule (blue) and Bβ12–46 (magenta) of another. The model was prepared with PYMOL [17] on the basis of the X-ray analysis data of chicken fibrinogen [18] and human D-dimer bound with synthetic peptide GPRP [19]**

While the fibrin protofibril formation mechanism is well-studied, the protofibril lateral association mechanism remains elusive. The initial interaction of fibrin monomers leading to protofibril formation is realized mainly by the pair of complementary centers A:a. An additional site «C» of protofibril formation is localized within the E domain [7]. The protofibril lateral association is realized by the lateral interactions of the D-D dimers from the neighboring protofibrils. The interacting fragments were determined as γ 350-360 and γ 370-380. The interprotofibril D-D dimer interactions lead to formation of so called D-tetramers [19].

However, Kollman *et al* [20] have discovered antiparallel contacts of fibrinogen coiled-coil fragments in the crystals of human fibrinogen and proposed the possibility of intermolecular coiled-coil interactions during the fibrin protofibril lateral association. We obtained earlier [21] the fibrin-specific mAb FnI-3c, which reacts with fibrin and does not react with fibrinogen and D-dimer. The NAD of this mAb formed during fibrinogen to fibrin transformation was localized preliminary within the BβM118-V133 fragment, which is the part of the coiled-coil connector of the fibrin(ogen) molecule. It was shown that mAb I-3c and its Fab-fragment specifically inhibit the stage of fibrin protofibril lateral association (Fig. 6). Thus, we suggested that this region also participates in the process of the fibrin protofibril lateral association [21].

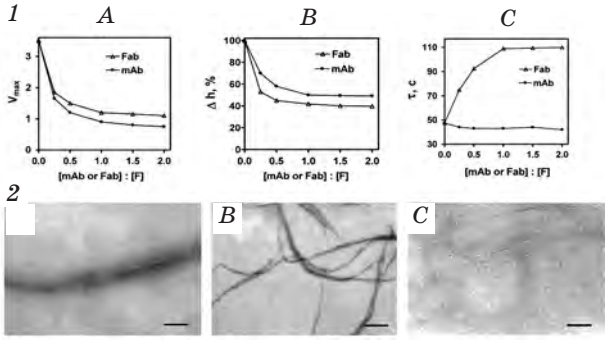

Fig. 6. **The influence of mAb FnI-3C and its Fab-fragment on polymerization of fibrin produced in fibrinogen+thrombin reaction in turbidity analysis (*1*). The dependence of the maximum rate of the fibrin polymerization V_{max} (*A*), final turbidity of fibrin clots Δh (*B*) and the lag time τ (*C*) on molar ratio of mAb FnI-3C or its Fab-fragment to fibrin. (*2*) Electron micrographs of negatively contrasted structures formed during polymerization of fibrin produced in fibrinogen+thrombin reaction in 135 s from start of the process: in the absence mAb FnI-3C or Fab-fragment (*A*); in the presence of mAb FnI-3C (*B*) or its Fab-fragment (*C*). The bars represent 200 nm**

Turbidity analysis and electron microscopy, showed that the synthetic peptide imitating fibrin fragment Bβ121-138, but not Bβ109-126, also inhibits the step of the fibrin protofibril lateral association. SPR analysis revealed that mAb FnI-3C interacts with the peptide Bβ121-138. This mAb also interacts with human, horse, and rabbit fibrins, but not with cow and rat fibrins. Alignment of the amino acid sequences of these fibrins allowed us to identify the residue BβK130 as crucial for the mAb FnI-3C epitope formation and to propose that the epitope in human fibrin is formed by the amino acid residues of the Bβ126-135 fragment. With the help of electrophoretic and ELISA parallel analysis we demonstrated that the exposition of the epitope is not the result of the removal of the αC-regions from the bulk of the molecule during the fibrinogen-fibrin transformation. SPR analysis reveals that the exposition takes place as the result of FpA splitting in a monomeric fibrin [22]. The analysis of the mutations in the of coiled-coil connector of the fibrin molecule corresponding to Bβ111-141, which impair fibrin polymerization, demonstrates that these mutations alter the structure of this fragment, but not its surface. Contrariwise, the mutations in the fragment Bβ158-170 of the coiled-coil connector alter the surface. The comparative conformational analysis of the fibrinogen and fibrin molecules suggests that the fragment of the coiled-coil connector corresponding to Bβ126-135 is more flexible in fibrin. The mobility of the fragments of the molecule around this fragment is higher in fibrin than in fibrinogen. Thus, we suppose that FpA splitting by thrombin leads to structural rearrangements in the human fibrin fragment of the coiled-coil connector, which includes polypeptide fragments Aα91-103, Bβ126-135 and γ69-77. These structural rearrangements provide fibrin protofibrils with a capability to associate laterally forming fibrils. The other fragment of the coiled-coil connector including Bβ158-170 may act as one of interprotofibril contact sites (Fig. 7).

MAb I-3C has been used as a «catch»-one in double-sandwich ELISA for soluble fibrin quantification in human blood plasma [18].

Monoclonal antibodies FnII-2M [23], I-5B and I-6B [24] to the αC-region of the human fibrin(ogen) have been obtained. The epitope for mAbs FnII-2M, I-5B are localized within the fibrin fragment Aα240-491 and for mAb I-6B — within Aα509-602 fragment. By virtue of the monAb FnII-2M, which react only with fibrin desAB and does not react with Fg and

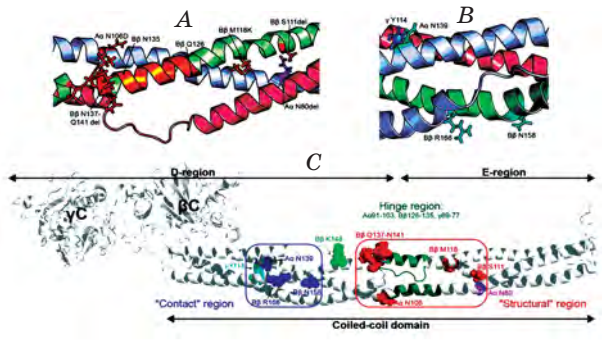

Fig. 7. A — **The localization of five human fibrinogen mutations in Bβ 111-141 and in the corresponding α-chain fragments that alter the structure of this coiled-coil fragment in mutant fibrinogens: Kyoto IV (Bβ S111del), Lyon (Bβ M118K), Epsom (Bβ N137- E141 del) and Plzen (Aα N106D) are shown as red sticks and Caracas VI (Aα N80del) is shown as magenta sticks. The suggested epitope for mAb FnI-3c (Bβ 126-135) is in orange.** ***B*** **— The localization of the amino acid residues where the structural alterations take place in the four mutant fibrinogens: Lima (Aα R141S), Niigata (Bβ N160S), Longmont (Bβ R166C) and San Diego II (γ Y114H) are shown as sticks. The novel sites Aα N139 and Bβ N158 of oligosaccharide attachment in fibrinogens Lima and Niigata are blue. The amino acid residue Bβ 166, where an individual cysteine from the medium attaches to Bβ 166C in fibrinogen Longmont (Bβ R166C), is blue. In fibrinogen San Diego II the buried hydrophobic Y114 (cyan) is replaced by H.** ***C*** **— The location of the SLA/NAD «structural» site corresponding to Bβ 111-141, the «contact» site corresponding to Bβ 158-170, and the «hinge region» (Aα 91-103, Bβ 126-135 and γ 69-77) in the human fibrin(ogen) molecule (PDB ID: 3GHG). The «structural» site and the hinge region are on the border between the D- and E-parts of the coiled-coil domain. The «contact» site is in the C- terminal part of the coiled-coil connector on the lateral side of the D-region of the molecule**

fibrin desA it was found that αC-regions are connected with FpB in the fibrinogen, monomer and polymer fibrin desA. However, the αC-regions move away from the core of the fibrin desA molecules after FpB cleavage by thrombin from fibrin desA in protofibrils, resulting in enhancing lateral association of protofibrils. It was been confirmed by ELISA and method of surface plasmon resonance (SPR) that monAb FnII-2M does not react with fibrinogen, monomeric and polymeric fibrin desA, but react with fibrin desAB. Thus, accessibility of this monAb to its epitope exists only in fibrin desAB. These results are direct experimental evidence that αC-regions (Aα220-610) are bound FpB in fibrinogen and fibrin desA molecules, but move away from the core of the molecule only after removal of

fibrinopeptides B (FpB) by thrombin. These data and the results of the other authors let us to design the three-dimensional structural computer models reflecting of αC-regions spatial orientation in fibrinogen, fibrin desA and fibrin desAB molecules (Fig. 8) [23].

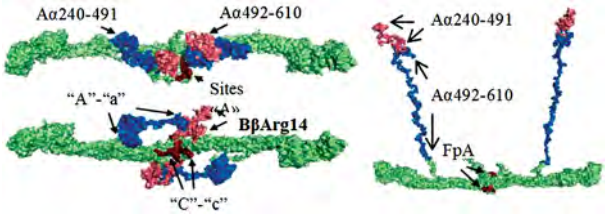

Fig. 8. **The models of fibrinogen, fibrin desA and fibrin desAB were done with PyMOL and Modeller9v10**

It was investigated that mAbs I-5B and I-6B decreased the maximum rate of fibrin desAB polymerization up to 90% and 76.2%, respectively, at their equimolar ratio to fibrin. These results suggest that these mAbs-inhibitors block the sites into αC-region, which take part in protein-protein interactions during the fibrin polymerization. The mAbs I-5B and I-6B may be used as tag-antibodies together with fibrin-specific «catch»-antibodies I- for quantification of the earliest forms of soluble fibrin in human blood plasma with the aim of early diagnostics of thrombophilia.

Soluble fibrin and D-dimer are the most specific markers of activation of the blood coagulation cascade and the threat of thrombosis. We have designed three immunoassay test-systems for D-dimer, soluble fibrin and fibrinogen quantification in human blood plasma on the basis of D-dimer-specific, fibrin-specific and fibrinogen-specific monoclonal antibodies. The forth test-systems was designed for simultaneous quantification of these molecular markers (Fig. 9). The clinical trials of the test systems were carried out in Ukraine. The high informativity of soluble fibrin quantification as a prognostic indicator of the threat of thrombosis at hip replacement (Fig. 10) and abdominal aorta (Fig. 11) was shown. Independent D-dimer quantification is uninformative. For the prediction of postoperative thrombotic complications and monitoring the efficiency of antithrombotic therapy the simultaneous quantification of soluble fibrin and D-dimer before the operation and at different time intervals after the operation is required. Only in this case it is possible to get information about the state of the balance between blood coagulation and fibrinolytic systems, and determine the degree of the threat of thrombosis.

Fig. 9. **The immunoassay test-systems for D-dimer, soluble fibrin and fibrinogen and its simultaneous quantification in human blood plasma designed by Palladin Institute of Biochemistry of NAS of Ukraine and LLC «Diaproph»**

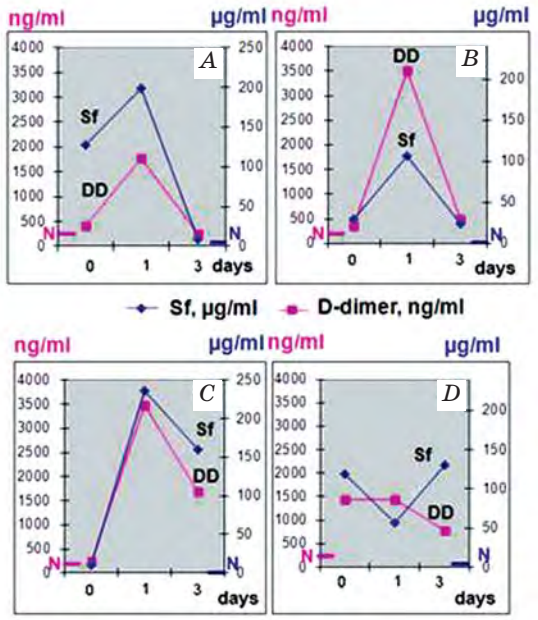

Fig. 10. **These figures show the typical combinations of the soluble fibrin (Sf) and D-dimer (DD) concentrations during aneurism of abdominal aorta before the operation, immediately after the operation and on the third day after the operation. Normal (*B, C*) or increased (*A, D*) concentrations of Sf and D-dimer were observed before the operation. After the operation in three cases (*A, B, C*) the significant increase of both markers was observed. On the third day after the operation in two of these cases (*A, B*) we can see the decrease of these concentrations to normal level. In the case (*C*) the concentrations of these markers also decreased, but remained above normal level, that required a further control. In the cases (*A, B, C*) there is a dynamic balance between blood clotting and fibrinolytic systems. It is illustrated by correlated concentration increase and decrease of both markers. In the case D: at once after the operation the concentration of Sf tended to decrease, and the concentration of D-dimer remained at the same level. But on the third day after the operation a steep increase of Sf and the decrease of D-dimer concentrations were observed. This suggests the disturbance of the balance between blood clotting and fibrinolytic systems and the high threat of thrombus formation**

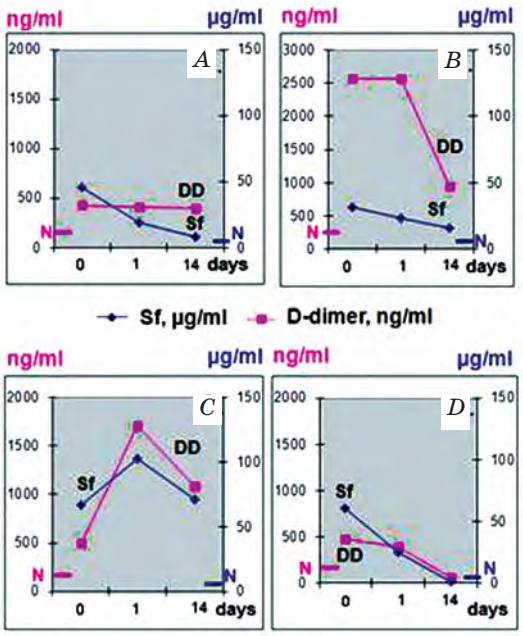

Fig. 11. **These figures show the typical combinations of the soluble fibrin (Sf) and D-dimer (DD) concentrations during deep vein thrombosis before the operation, immediately after the operation and on the 14 day after the operation. The main feature of this disease is high concentrations of both markers before treatment that indicates the high degree of activation of blood clotting and fibrinolytic systems. This suggests that during this pathology the activation of blood clotting system as a rule leads to the activation of fibrinolytic system. The dynamics of the concentration changes of these markers are usually correlated: we can see simultaneous increase (*C*) or decrease (*A*, *B*, *D*) of the concentrations of both markers during treatment. In the case (*C*) the correlated changes of the concentrations of these markers also takes place, but the high concentrations of these markers on the 14 day after operation testify about necessity of further control**

A hundred of mAbs against neoantigenic determinants of fibrinogen, fibrin and their fragments were obtained. In this review we focused on 14 of them, that were used to obtain the new fundamental scientific information or to develop immunodiagnostic test-systems, which have strategic importance for diagnostics of the threat of thrombus formation.

The arrows at the Fig. 12 point at the sites of fibrinogen and fibrin molecules, the mAbs are directed to. Thus, mAb 2d-2a was obtained against the epitope, which consists of amino acid residues located around the peptide bond Bβ14-15 cleaved by thrombin. This MAb reacted with fibrinogen and fibrin desA but doesn't with fibrin desAB. These molecules keep the fibrinopeptides B uncleaved. Using 2d-2a mAb we showed for the first time the existence of the site of fibrin protofibrils lateral association within the BβN-domain. This site functions before fibrinopeptides B cleavage off. This work was continued and 4 other mAbs against BβN-domain (Bβ1-64) were obtained. Two of them were directed to site Bβ15-25, and others to Bβ26-36. Using these mAbs we discovered that there is not only the site of the protofibrils lateral association in the region of BbN-domain, but also the site, which takes a part in the first stage of fibrin polymerization — protofibrils formation.

MAb III-3b obtained in this work reacts only with D-dimer, but doesn't with fibrinogen and fibrin. By virtue of this mAb test-system for quantification of D-dimer in human blood plasma was developed to diagnose the hemostasis disorders. Other MAb II-4d allowed us to locate the fibrin polymerization site in the NH_2-terminal part of γ-chain of D-region, which does not coincide with the

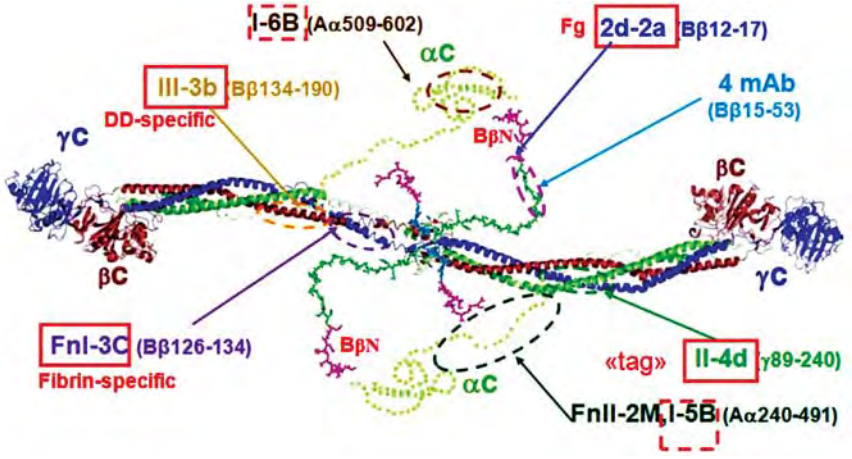

Fig. 12. **The model of fibrinogen molecule, where are shown the mAbs and localization of epitopes for them**

polymerization site «a». MAb II-4d, which reacts with fibrinogen, soluble fibrin and D-dimer, has been used for the design of three immunodiagnostic test-systems for quantification of these molecular markers of thrombophilia in human blood plasma as the tag-antibody.

By virtue of fibrin-specific mAb FnI-3C we discovered an neoantigenic determinant in fibrin molecule, which is exposed after the conversion of fibrinogen into fibrin. This determinant is located in Bβ126-135 and coincides with the epitope for mAb I-3C and with the site, which plays functional role in the fibrin protofibrils lateral association. Using this mAb the functional role of coiled-coil connector of fibrin molecule in the fibrin polymerization was showed for the first time. MAb I-3C reacts only with fibrin, does not react with fibrinogen and D-dimer and can be used in double-sandwich ELISA test-system for soluble fibrin quantification in human blood plasma as a «catch»-mAb for diagnostics of the threat of thrombus formation.

Finally, three mAbs to αC-connector of fibrinogen were produced. MAb II-2M of IgM class allowed us to confirm binding of αC-connector to fibrinopeptides B in fibrinogen and fibrin desA molecules. The presence of two polymerization sites in C-terminal parts of αC-connector was also shown using other mAbs I-5B and I-6B.

Thus monoclonal antibodies proved to be very useful for discovering unknown antigenic determinants and polymerization sites within the fibrin molecule; for the study of the three-dimensional structure of the fibrin molecule and for designing of test-systems to diagnose the threat of the intravascular thrombus formation.

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ВИКОРИСТАННЯ МОНОКЛОНАЛЬНИХ АНТИТІЛ ДЛЯ ВИЗНАЧЕННЯ ЛОКАЛІЗАЦІЇ АНТИГЕННИХ ДЕТЕРМІНАНТ І САЙТІВ ПОЛІМЕРИЗАЦІЇ ФІБРИНУ ВСЕРЕДИНІ МОЛЕКУЛ ФІБРИНОГЕНУ ТА ФІБРИНУ І ЇХ ЗАСТОСУВАННЯ У ТЕСТ-СИСТЕМАХ ДІАГНОСТИКИ ТРОМБОУТВОРЕННЯ

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Із використанням моноклональних антитіл було показано, що BβN-регіон молекули фібрину desA (Bβ1-53) містить сайт полімеризації, що включає пептидний зв'язок Bβ14-15, який бере участь у другій стадії полімеризації фібрину — латеральній асоціації протофібрил.

ИСПОЛЬЗОВАНИЕ МОНОКЛОНАЛЬНЫХ АНТИТЕЛ ДЛЯ ОПРЕДЕЛЕНИЯ ЛОКАЛИЗАЦИИ АНТИГЕННЫХ ДЕТЕРМИНАНТ И САЙТОВ ПОЛИМЕРИЗАЦИИ ФИБРИНА ВНУТРИ МОЛЕКУЛ ФИБРИНОГЕНА И ФИБРИНА И ИХ ПРИМЕНЕНИЕ В ТЕСТ-СИСТЕМАХ ДИАГНОСТИКИ ТРОМБООБРАЗОВАНИЯ

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С использованием моноклональных антител было показано, что BβN-регион молекулы фибрина desA (Bβ1-53) содержит сайт полимеризации, включающий пептидную связь Bβ14-15, которая принимает участие во второй стадии полимеризации фибрина —

У фрагменті Bβ15-53 також виявлено сайт, названий «С», який разом із сайтом «А» бере участь у першій стадії полімеризації — утворенні протофібрил. Створено модель первинної міжмолекулярної взаємодії фібрину. В N-кінцевій половині γ-ланцюга D-регіону фібрину із застосуванням моноклональних антитіл II-4d було виявлено сайт («с»), який бере участь у побудові протофібрил і, за нашим припущенням, є комплементарним сайту «С».

Знайдено дві неоантигенні детермінанти, одна з яких експонується в суперспіральному фрагменті фібрину Bβ126-135 в результаті відщеплення фібринопептидів А від фібриногену тромбіном. Зміна просторової структури молекули фібрину, що її виявлено в цьому сайті, є необхідною для здійснення подальшої латеральної асоціації протофібрил. Друга неоантигенна детермінанта формується у фрагменті Bβ134-190 D-димеру, який утворюється під час розщеплення фібрину, стабілізованого FXIIIa, плазміном. Проти першої детермінанти отримано фібринспецифічне моноклональне антитіло FnI-, а проти другої — D-димер-специфічне III-3b.

Одержано 3 моноклональних антитіла проти αC-регіону молекули фібрин (оген)у. За допомогою одного з них було експериментально показано, що αC-домен з'єднаний з фібринопептидом В у фібриногені та у фібрині desA. Цей домен відходить від остова молекули після відщеплення фібринопептидів В тромбіном. Два інших моноклональних антитіла специфічно інгібують полімеризацію фібрину блокуванням двох невідомих сайтів полімеризації в αC-регіоні.

На основі моноклональних антитіл FnI- і III-3b, використовуваних як «catch»-антитіла, і антитіл II-4d — як «tag»-антитіла розроблено тест-системи для кількісного визначення розчинного фібрину та D-димеру в плазмі крові людини. Ці тест-системи апробовано в клінічних випробуваннях в Україні. Показано, що для прогнозування післяопераційних тромботичних ускладнень, а також для контролю ефективності антитромботичної терапії є необхідним одночасне кількісне визначення розчинного фібрину та D-димеру до операції та в різні періоди після операції. Тільки в цьому разі можна отримати інформацію про стан балансу між системами зсідання крові та фібринолізу і визначити ступінь загрози тромбоутворення.

Ключові слова: фібриноген, фібрин, моноклональні антитіла, тромбоутворення, діагностичні тест-системи.

латеральной ассоциации протофибрилл. Во фрагменте Bβ15-53 также обнаружен сайт, названный «С», который вместе с сайтом «А» принимает участие в первой стадии полимеризации — образовании протофибрилл. Создана модель первичного межмолекулярного взаимодействия фибрина. В N-концевой половине γ-цепи D-региона фибрина с применением моноклональных антител II-4d был обнаружен сайт («с»), который принимает участие в построении протофибрилл и, по нашему предположению, комплементарен сайту «С».

Обнаружены две неоантигенные детерминанты, одна из которых экспонируется в суперспиральном фрагменте фибрина Bβ126-135 в результате отщепления фибринопептидов А от фибриногена тромбином. Изменение пространственной структуры молекулы фибрина, выявленное в этом сайте, необходимо для осуществления последующей латеральной ассоциации протофибрилл. Вторая неоантигенная детерминанта формируется во фрагменте Bβ134-190 D-димера, образующемся при расщеплении фибрина, стабилизированного FXIIIa, плазмином. Против первой детерминанты получено фибринспецифическое моноклональное антитело FnI-, а против второй — D-димер-специфическое III-3b.

Получены 3 моноклональных антитела против αC-региона молекулы фибрин(оген)а. С помощью одного из них было экспериментально показано, что αC-домен соединен с фибринопептидом В в фибриногене и в фибрине desA. Этот домен отходит от остова молекулы после отщепления фибринопептидов В тромбином. Два других моноклональных антитела специфически ингибируют полимеризацию фибрина путем блокировки двух неизвестных сайтов полимеризации в αC-регионе.

На основе моноклональных антител FnI- и III-3b, используемых в качестве «catch»-антител, и антител II-4d — в качестве «tag»-антител разработаны тест-системы для количественного определения растворимого фибрина и D-димера в плазме крови человека. Эти тест-системы апробированы в клинических испытаниях в Украине. Показано, что для прогнозирования послеоперационных тромботических осложнений, а также для контроля эффективности антитромботической терапии необходимо одновременное количественное определение растворимого фибрина и D-димера до операции и в разные периоды после операции. Только в этом случае можно получить информацию о состоянии баланса между системами свертывания крови и фибринолиза и определить степень угрозы тромбообразования.

Ключевые слова: фибриноген, фибрин, моноклональные антитела, тромбообразование, диагностические тест-системы.