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C-Terminal Fragment of Human Laminin-Binding Protein Contains a Receptor Domain for Venezuelan Equine Encephalitis and Tick-Borne Encephalitis Viruses

A. A. Malygin[1], E. I. Bondarenko[2], V. A. Ivanisenko[3], E. V. Protopopova[2], G. G. Karpova[1], and V. B. Loktev[2]*

[1]*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, pr. Akademika Lavrent'eva 8, 630090 Novosibirsk, Russia; fax: (383) 333-3677; E-mail: karpova@niboch.nsc.ru*
[2]*State Research Center of Virology and Biotechnology "Vector", 630559 Koltsovo, Novosibirsk Region, Russia; fax: (383) 336-7409; E-mail: loktev@vector.nsc.ru*
[3]*Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, pr. Akademika Lavrent'eva 10, 630090 Novosibirsk, Russia; fax: (383) 333-1278; E-mail: salix@bionet.nsc.ru*

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Abstract—Polyclonal and monoclonal antibodies (MABs) to human laminin-binding protein (LBP) can efficiently block the penetration of some alpha- and flaviviruses into the cell. A panel of 13 types of MABs to human recombinant LBP was used for more detailed study of the mechanism of this process. Competitive analysis has shown that MABs to LBP can be divided into six different competition groups. MABs 4F6 and 8E4 classified under competition groups 3 and 4 can inhibit the replication of Venezuelan equine encephalitis virus (VEEV), which is indicative of their interaction with the receptor domain of LBP providing for binding with virions. According to enzyme immunoassay and immunoblotting data, polyclonal anti-idiotypic antibodies to MABs 4F6 and 8E4 modeling paratopes of the LBP receptor domain can specifically interact with VEEV E2 protein and tick-borne encephalitis virus (TBEV) E protein. Mapping of binding sites of MABs 4F6 and 8E4 with LBP by constructing short deletion fragments of the human LBP molecule has shown that MAB 8E4 interacts with the fragment of amino acid residues 187-210, and MAB 4F6 interacts with the fragment of residues 263-278 of LBP protein, which is represented by two TEDWS peptides separated by four amino acid residues. This suggested that the LBP receptor domain interacting with VEEV E2 and TBEV E viral proteins is located at the C-terminal fragment of the LBP molecule. A model of the spatial structure of the LBP receptor domain distally limited by four linear loops (two of which are represented by experimentally mapped regions of amino acid residues 187-210 and 263-278) as well as the central β-folded region turning into the α-helical site including residues 200-216 of the LBP molecule and providing for the interaction with the laminin-1 molecule has been proposed.

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Key words: laminin-binding protein, receptor domain, anti-idiotypic antibodies, peptide, TEDWS, Venezuelan equine encephalitis virus, tick-borne encephalitis virus

Laminin-binding protein (LBP) (laminin receptor) is a member of the non-integrin protein family [1]. Interaction of this protein with laminins on the cell surface provides for growth and formation of tissues and organs as well as for correct cell differentiation [2]. The LBP gene is a member of the family of steadily expressed cell genes, and the disturbance of its functioning induces cell apoptosis [3]. It encodes a precursor polypeptide of 295 amino acids with a calculated molecular mass of ~32 kDa [4]. The amino acid sequence of LBP is rather conservative with 98.3-99.0% homology level for mice, rats, cows, and humans [5]. Homologs of this protein have been found in bacteria, fungi, plants, and animals, which suggests its presence in practically all organisms. The electrophoretic mobility of the precursor polypeptide corresponds to molecular mass from 37 to 45 kDa. Most

Abbreviations: AIA, anti-idiotypic antibodies; (C)EIA, (competitive) enzyme immunoassay; MABs, monoclonal antibodies; RD, receptor domain; (r)LBP, (recombinant) laminin-binding protein; TBEV, tick-borne encephalitis virus; VEEV, Venezuelan equine encephalitis virus; XRDA, X-ray diffraction analysis.

* To whom correspondence should be addressed.

probably, the formation of the dimeric form of the molecule with a molecular mass of 67 kDa occurs by means of acylation, and mainly 67 kDa LBP is surface exposed [6].

The peculiarities of the LBP molecule should include the absence of potential glycosylation sites and the presence of a hydrophobic domain at 2/3 of the distance from the N-terminal part of the polypeptide [5]. The N-terminal part of LBP, residues from 1 to 85, is located in the cytosolic part of the cell, and the sequence of residues from 86 to 101 represents the putative membrane domain of LBP. The C-terminal part of LBP is on the external surface of the cell membrane. It was shown that the sequences of residues from 161 to 180 and from 205 to 229 provide for the interaction between LBP and the laminin-1 molecule [7, 8]. The YIGSR pentapeptide from the laminin-1 molecule can interact with the 67 kDa LBP with high affinity. Recently, the results of X-ray diffraction analysis (XRDA) of LBP fragment 1-220 with resolution of 2.15 Å have been published [9]. LBP was shown to have a globular structure comprising five α-helical and seven β-folded regions.

Besides cell membrane, LBP can also be found in the cell nucleus where it interacts with histone proteins [10]. LBP was also detected in the 40S ribosomal subunit and was named p40 protein [11]. Unlike the levels of most ribosomal proteins, p40 level in subunits is not constant and changes depending on the translation level. During active protein synthesis, the p40 level in ribosomes increases; it decreases with general reduction of translational activity in the cell [12]. Not long ago, the eukaryote-specific C-terminal domain of p40 protein was shown to be involved in binding with 40S subunits [13]. In addition, this protein participates in the organization of a binding site for the specific structural element of hepatitis C virus RNA, the so-called IRES-element, on the 40S subunit [14].

Increased LBP expression is the determining and controlling factor of metastatic spread of some cancers [1]. Prion proteins use LBP as a cell receptor to penetrate into cells, which accounts for the important role of LBP in the pathogenesis of prion diseases [15, 16]. Some viruses also use LBP as a cell receptor, such as tick-borne encephalitis (TBEV), dengue, West Nile, Sindbis, and Venezuelan equine encephalitis (VEEV) viruses [17-23].

Our previous research confirmed the hypothesis that VEEV penetrates into target cells using LBP as a receptor [24-26]. Human recombinant LBP (rLBP) was shown to effectively react with purified VEEV virions. Polyclonal rabbit antibodies to VEEV effectively inhibit the interaction of rLBP with VEEV virions in competitive enzyme immunoassay (EIA), which also confirms the specificity of the interaction between LBP and viral proteins. Treating Vero cells with polyclonal antibodies to rLBP inhibits VEEV replication in them by more than 300,000 times. Monoclonal antibodies (MABs) to rLBP also proved to be capable of binding with LBP on the cell surface, simultaneously inhibiting VEEV replication [25, 27].

The goal of the present work was to map the receptor region of LBP providing its interaction with alpha- and flaviviruses. Human rLBP, its short deletion fragments, a panel of MABs to human LBP, and anti-idiotypic antibodies (AIA) modeling LBP epitopes were used for this purpose.

MATERIALS AND METHODS

Cell cultures and viruses. Vero cell cultures, PEK, and RH were obtained from the Cell Culture Bank of State Research Center of Virology and Biotechnology (SRC VB) "Vector" and supported on Eagle's MEM containing 7-10% fetal calf serum and 80 μg/ml gentamicin sulfate. Venezuelan equine encephalitis virus (VEEV), strain TC-83, obtained from the collection of SRC VB "Vector" was used in the work. The virus was cultivated on Vero cell culture using DMEM medium containing 2% bovine serum and 80 μg/ml of gentamicin sulfate. Infectious activity of viral suspensions was determined by titration in 96-well plates by the CPE development on Vero cell culture [28]. Aliquots of the viral suspension were stored at −70°C.

Tick-borne encephalitis virus (TBEV), strain 205, was also used in the work; viral material was obtained from the State Viral Strains Collection of the D. I. Ivanovsky Institute of Virology, Russian Academy of Medical Sciences. TBEV was cultivated on PEK and/or RH cell cultures. VEEV and TBEV were purified in sucrose density gradients as described in [29]. Protein concentration was determined with the Bio-Rad (USA) Protein Assay Kit according to the manufacturer's instructions using bovine serum albumin as a standard.

rLBP and its deletion forms. Production, purification, and the main properties of rLBP are described in [30]. LBP cDNA fragments were produced by PCR according to the technique described in [31] using pairs of primers having sequences for creation of restriction sites *Nde* I and *Bam*H I in PCR product at their 5′-ends. Oligonucleotide 5′-GAGAATTCCATATGTCCGGAGCCCTTGAT-3′ was used as a "forward" primer. The following oligonucleotides were used as "reverse" primers—oligonucleotide 5′-GAGAATTCGGATCCTTAAGACCAGTCAGTGGTT-3′ in the case of full-length LBP and oligonucleotides 5′-GAGGATCCTTAAATCTCTTCAGGATCTCT-3′, 5′-GAGGATCCTTAAGCGGGAGCAGTCCATTG-3′, 5′-GAGGATCCTTAGAATTGCTGAATAGGCAC-3′, 5′-GAGGATCCTTATGCAGACCAGTCTTCCGT-3′, and 5′-GAGAATTCGGATCCTTAACGCATGCGCAGAACTT-3′ in the case of its shortened forms LBP Δ279-295, LBP Δ263-295, LBP Δ236-295, LBP Δ211-295, and LBP Δ187-295, respectively. After treatment with restrictases *Nde* I and *Bam*H

I, PCR products were cloned into vector pET15b (Novagen, USA) at the same restriction sites. The resulting plasmids were expressed in *Escherichia coli* cells, strain BL-21(DE-3). To produce recombinant proteins, the cell were grown in LB medium in the presence of 100 mg/liter of ampicillin at 37°C and active stirring to achieve optical density A_{600} = 0.6. Recombinant protein synthesis was induced by adding isopropyl-D-thiogalactopyranoside to the culture to the concentration of 0.4 mM followed by incubation for 3 h more. After the incubation, 500-μl aliquots were collected from cell cultures, the cells were deposited, lysed with 1% SDS at 95°C for 15 min, and the lysate was applied to 14% SDS-polyacrylamide gel to separate proteins by gel electrophoresis. After separation, the proteins were transferred to nitrocellulose membrane and stained with Ponceau S (Sigma, USA). Immunoblotting was performed using MABs 4F6 and 8E4; bound antibodies were detected with IgG to murine antibodies conjugated with horseradish peroxidase (Sigma) and with the ECL visualization kit (Pierce, USA).

Polyclonal and monoclonal antibodies. The collection of murine MABs and polyclonal antibodies to rLBP used in this work was described by us previously [27, 30]. Polyclonal and monoclonal antibodies to VEEV and TBEV were produced according to the methods described in [32]. Antibodies were purified with caprylic acid followed by desalination of immunoglobulins with semi-saturated ammonium sulfate solution [33]. The protein concentration was measured with the Bio-Rad Protein Assay Kit using purified rat IgG to construct the calibration curve. MAB was biotinylated as described in [34].

Production of polyclonal rabbit AIA. Rabbit AIA were produced by fourfold immunization of animals via subcutaneous administration of 5-10 mg of a mixture of murine MABs 4F6 and 8E4 at two-week intervals. Antigen was introduced in a mixture with an equal volume of complete (first immunization) and later of incomplete Freund's adjuvant (Sigma).

Enzyme immunoassay. Purified VEEV or TBEV (2 μg/ml) were sorbed in 100 μl of 0.05 M phosphate buffer, pH 8.0, in 96-well plates (Medpolymer, Russia) overnight at 4°C. Nonspecific binding sites in polystyrene plate wells were blocked by adding 0.25% egg albumin solution (ICN, USA) in 145 mM NaCl, 10 mM Tris-HCl, 0.5% phenol red, and 0.1% Tween-20, pH 7.4 (Tris-buffered saline, TBS). Rabbit serum containing AIA at different dilutions in 0.2% egg albumin solution in TBS was added into the wells followed by incubation for 1 h at 37°C. The plates were washed 5 times in TBS. Immune complexes were detected with anti-species peroxidase conjugate. After washing 3 times with TBS, *o*-phenylenediamine solution (1 mg/ml *o*-phenylenediamine, 0.03% hydrogen peroxide) in citrate-phosphate buffer (0.2 M citric acid and 0.5 M Na_2HPO_4, pH 5.0) was added into the wells. The plates were kept in this solution for 30 min, and then the staining reaction was stopped by adding 1 N hydrochloric acid (100 μl per well). Optical density of samples was measured on spectrophotometer (Uniscan, Finland) at the wavelength of 492 nm. Samples with OD values exceeding the negative control by three times were considered to be positive.

Immunoblotting. Western blotting was performed according to [35] after separation of proteins by electrophoresis in 7.5% polyacrylamide gel for VEEV lysates and in 12% gel for TBEV according to Laemmli [36]. The forming immune complexes were detected with anti-species peroxidase conjugate.

Competitive enzyme immunoassay (CEIA). rLBP (2 μg/ml) was sorbed in 96-well plates as described above. After washing and blocking nonspecific binding sites, antigen was incubated with purified MABs or polyclonal antibodies to rLBP for 12 h at 4°C. Then, after washing 5 times, biotin-labeled MABs in dilutions selected individually for each labeled MAB by preliminary titration were added to the wells. After incubation for 1 h, unbound labeled antibodies were washed away, and the forming immune complexes were detected with streptavidin-peroxidase for 20 min followed by stopping the reaction with 1 N HCl. Specific binding of labeled antibodies with rLBP was determined as described above. The presence of competition was revealed by decreased efficiency of binding of labeled MABs with rLBP after incubating it with other unlabeled antibodies. The signal of binding of labeled MAB with rLBP without preliminary incubation with other antibodies was taken as 100%. The competition level was evaluated by four gradations: complete competition (unlabeled antibodies inhibit the binding of labeled MABs with antigen by more than 75%), partial competition with a maximal effect (unlabeled antibodies inhibit binding by 50-75%), partial competition with a minimal effect (unlabeled antibodies inhibit binding by more than 25%, but less than 50%), and the absence of competition (inhibition of binding by less than 25%).

Analysis of spatial structure of the receptor region. The spatial structure of the receptor region was modeled using the 3D-Jury metaserver, which automatically uses free servers for prediction of the tertiary structure of proteins through the internet [37]. The structure predicted by server FFAS03: Fold & Function Assignment System [38] was selected for further analysis of predictions as the most complete one and consistent with the results of secondary structure predictions. Coordinates of atoms were obtained with the Modeller program [39]. The spatial structure of cgd2_2020 protein (PDB Id 2pd0) was used as a 3D structure pattern for the analyzed fragment of the LBP molecule. To model the spatial structure of the site of rLBP sequence (262-277) containing the TEDWS repeat, the spatial structure of the loop (118-133) of IIIA CBD protein (PDB Id 1g43) was additionally used. The secondary structure was predicted using the programs Jpred [40], Porter [41], PHD [42], and GOR V [43]. In

addition, the preference profiles of amino acid residues of the LBP sequence for the formation of the secondary structure were constructed to evaluate the secondary structure [44].

RESULTS

Monoclonal antibodies to human LBP. Previously we obtained a panel of 13 types of murine MABs to human LBP [27]. A competitive solid-phase EIA using biotin-labeled and unlabeled MABs was performed in the present work for detailed mapping of binding epitopes for monoclonal antibodies on LBP. MABs were divided into six groups based on the analysis of mutual competition of antibodies for binding with rLBP (table). Five competition groups (12 of 13 MAB types) partially overlapped and combined not less than nine different epitopes, and MAB 3E6 corresponded to an individual competition group comprising a single epitope. Polyclonal antibodies to rLBP completely blocked binding of all MAB types with rLBP, which provided additional evidence for the specificity of interaction between antibodies and LBP.

Previously it was found that polyclonal rabbit antibodies to LBP and murine MABs 4F6, 8E4, and 10E4 can interact with LBP on the surface of Vero cells and inhibit VEEV replication in these cells [24-26]. Monoclonal antibodies 9D8 can also inhibit VEEV replication on Vero cell culture (data not given). The competitive analysis data show that MABs 4F6 and 10E4 of the 3rd competition group and MABs 8E4 and 9D8 of the 4th competition group partially compete with each other (table). This suggested that these four MAB types recognize the active center of the LBP molecule providing for the interaction between virions and LBP molecules on the cell surface, which results in the inhibition of VEEV replication in Vero cells. The absence of biological activity in other types of MABs to LBP additionally confirms the specificity of this interaction. MABs 10E4 and 9D8 were typed as class IgM immunoglobulins, MABs 8E4 as

Competition of MABs for interaction with rLBP in CEIA

Unlabeled MABs	Competition group	Biotin-labeled MABs									
		3G5	21H1	16A3	1B5	2H9	1D2	**4F6**	**10E4**	**8E4**	**9D8**
3G5	1	+++	++	–	–	–	–	–	–	–	–
21H1		++	+++	++	++	+	++	++	+	–	–
16A3	2	–	++	+++	++	+	++	++	+	–	–
1B5		–	++	++	+++	+	–	+	++	–	–
2H9		–	+	+	+	+++	–	–	+	+	–
1D2		–	++	++	–	–	+++	–	–	–	–
4F6	3	–	++	++	+	–	–	+++	++	+	++
10E4		–	+	+	++	+	–	+	+++	++	+
8E4	4	–	–	–	–	+	–	+	++	+++	–
9D8		–	–	–	–	–	–	++	++	–	+++
7A5	5	–	–	–	–	++	++	–	+	–	–
4C11		–	–	–	–	++	++	–	+	–	–
3E6	6	–	–	–	–	–	–	–	–	–	–
Rabbit antibodies to rLBP		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Normal mouse serum		–	–	–	–	–	–	–	–	–	–

Note: +++, inhibition of binding of labeled MABs with rLBP by unlabeled antibodies by more than 75%; ++, corresponds to inhibition of binding by 50-75%; +, inhibition of binding by 25-50%; –, inhibition of binding by less than 25%.

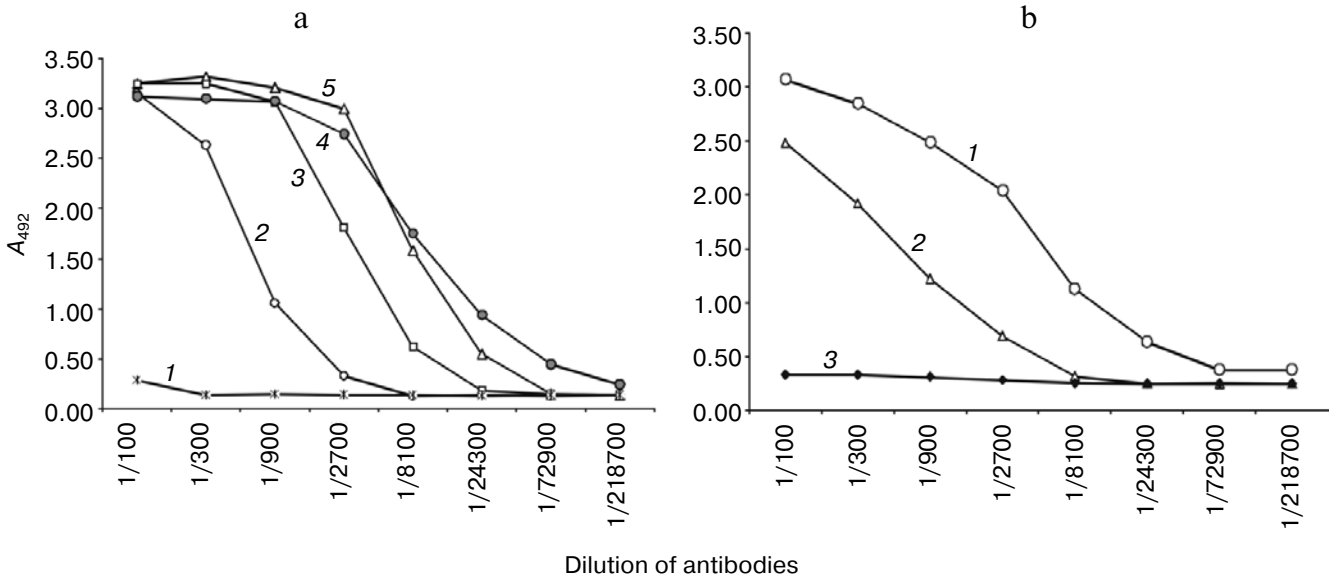

Fig. 1. Interaction of rabbit AIA to MABs 4F6 and 8E4 with purified VEEV and TBEV virions in EIA. a) VEEV: *1*) negative control with normal rabbit serum; *2-4*) with rabbit AIA obtained as result of two-, three-, and fourfold immunization with MABs 4F6 and 8E4; *5*) positive control with polyclonal rabbit antibodies to VEEV. b) TBEV: *1*) positive control with MAB 10H10 to TBEV (strain 205); *2*) interaction with AIA to MABs 4F6 and 8E4 (fourfold immunization); *3*) negative control with normal rabbit serum.

class IgG1, and 4F6 as class IgG2a immunoglobulins [27]. That is why MABs 8E4 and MABs 4F6 were used for further experiments on mapping the LBP receptor region interacting with virions.

Anti-idiotypic antibodies to MABs 4F6 and 8E4. If MABs 4F6 and 8E4 really recognize the active center of LBP, AIA for these antibodies should have paratopes that model LBP epitopes providing for specific interaction with VEEV virions. Polyclonal rabbit AIA to MABs 4F6 and 8E4 classified under two partially overlapping competition groups and having the ability to block virus replication in sensitive cells were produced to verify this supposition. AIA obtained after fourfold immunization with a mixture of purified MABs 4F6 and 8E4 effectively interacted with VEEV in EIA (Fig. 1a). Besides, the AIA were found to effectively bind with purified TBEV virions (Fig. 1b). This satisfactorily conforms to the fact that TBEV also uses LBP as a cell receptor [17]. Western blotting confirmed the specificity of these two interactions showing that AIA interact with the surface VEEV E2 glycoprotein (strain TC-83) and TBEV E glycoprotein (Fig. 2). These results indicate that MABs 4F6 and 8E4 recognize epitope(s) on the surface of the LBP molecule that can specifically interact with TBEV E protein and VEEV E2 protein.

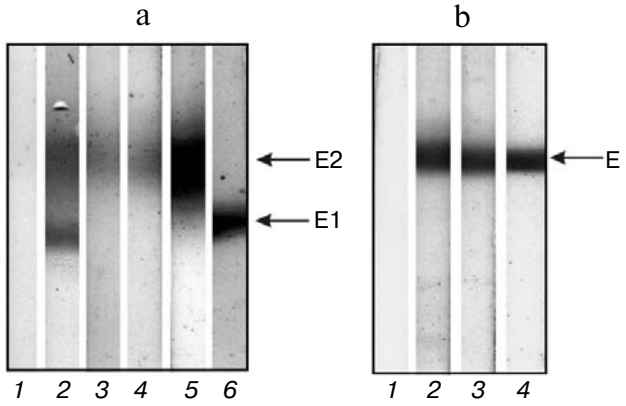

Fig. 2. Immunoblotting of purified VEEV and TBEV suspension. a) VEEV: *1*) negative control with normal rabbit serum diluted 1 : 1000; *2*) strip treated with polyclonal rabbit serum to VEEV, strain TC-83, diluted 1 : 2000; *3*) rabbit AIA to 4F6 and 8E4 diluted 1 : 1000; *4*) rabbit AIA to 4F6 and 8E4 diluted 1 : 2000; *5*) murine MAB 1D10 to VEEV E2 protein (1 : 2000); *6*) murine MAB 2E12 to VEEV E1 protein (1 : 200). b) TBEV: *1*) negative control with normal rabbit serum diluted 1 : 500; *2*, *3*) strips treated with AIA diluted 1 : 500 and 1 : 1000, respectively; *4*) murine MAB 13F6 to TBEV E protein diluted 1 : 500.

Mapping the binding epitopes for MABs 4F6 and 8E4 on the LBP molecule. MABs 4F6 and 8E4 and shortened LBP forms containing deletions of different lengths in the C-terminal part of the protein were used to determine binding sites with VEEV and TBEV on LBP. Five shortened LBP forms, along with the full-length protein, were obtained via expression of the corresponding plasmid constructions in *E. coli* cells; the proteins were separated by SDS-PAGE, and after transfer onto nitrocellulose membrane were treated with MABs 4F6 and 8E4 (Fig. 3). MAB 8E4 was found to bind to all the used deletion variants of LBP except for the polypeptide containing a deletion of the fragment of residues from 187 to 295. As all

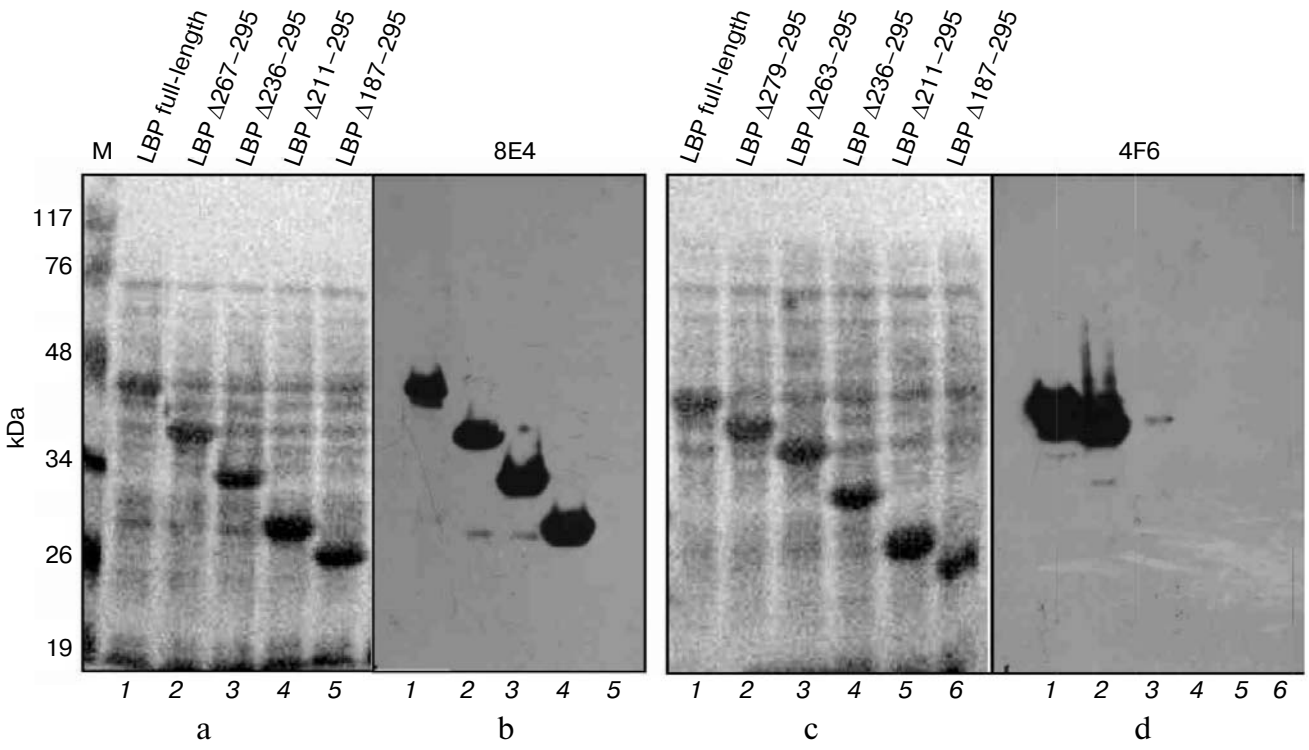

Fig. 3. Determination of epitopes for MABs 4F6 and 8E4 on the LBP molecule. a, c) Membranes after electrophoretic separation and transfer of proteins from lysates of cells producing recombinant LBP (full-length LBP bands) and its shortened forms (bands LBP Δ279-295, LBP Δ263-295, LBP Δ236-295, LBP Δ211-295, and LBP Δ187-295). M, protein marker (protein mass in kDa is indicated on the left). b, d) Membranes (as described in (a, c)) after binding with MABs 8E4 and 4F6, respectively, and detections of antibodies with ECL.

longer deletion forms of the protein including the polypeptide with a deletion of the fragment of residues from 211 to 295 interacted with MAB 8E4, it can be stated that the epitope for these antibodies is in the region of residues 187-210 of LBP (Fig. 3). MAB 4F6 interacted only with the full-length LBP molecule and the polypeptide containing a deletion of the C-terminal fragment of residues from 279 to 295. The binding of MAB 4F6 was not observed with shorter LBP forms lacking the C-terminal fragment beginning from residue 263. Thus, the epitope for MAB 4F6 is located in LBP in the region between residues 263 and 278 (Fig. 3). It should be noted that this region of LBP contains a sequence consisting of two identical pentapeptides (TEDWS) separated by only four amino acid residues.

Modeling of spatial structure of the receptor region of LBP. Computer modeling of the receptor region of LBP allowed us to propose a model of spatial structure of this region (Fig. 4). The model includes an expressed β-folded site converted into α-helical site and comprising residues 200-216 of the LBP molecule. This region is surrounded by four linear loops, two of which include LBP fragments 187-210 and 263-278 experimentally mapped in the present work. During modeling, we paid special attention to the structure of the site of residues 263-278 containing a TEDWS repeat. Such a repeat was found in the sequence of IIIA CBD protein from *Clostridium cellulolyticum*, for which the spatial structure is known (PDB Id 1g43). The repeat was located in the loop site (residues 118-133) of this protein. The structure of the rLBP loop (residues 263-278) predicted by modeling has a similar conformation with the corresponding loop of IIIA CBD protein.

DISCUSSION

The results of X-ray diffraction analysis (XRDA) of the LBP fragment 1-220 with the resolution of 2.15 Å have been published recently [9]. LBP was shown to have globular structure comprising five α-helical and seven β-folded regions. However, the spatial structure of the greater part of the protein receptor region (residues 221-295) remains unknown.

Our data show that MABs 8E4 and 4F6 can interact with comparatively short amino acid sequences of residues 187-210 and 263-278 of LBP, respectively. The proposed binding epitope for MAB $8E4_{187\text{-}210}$ is located between two canonical peptides providing for the LBP and laminin-1 interaction [5]. This is the so-called peptide G including amino acid residues 161-180 and providing for heparin-dependent interaction of LBP with a

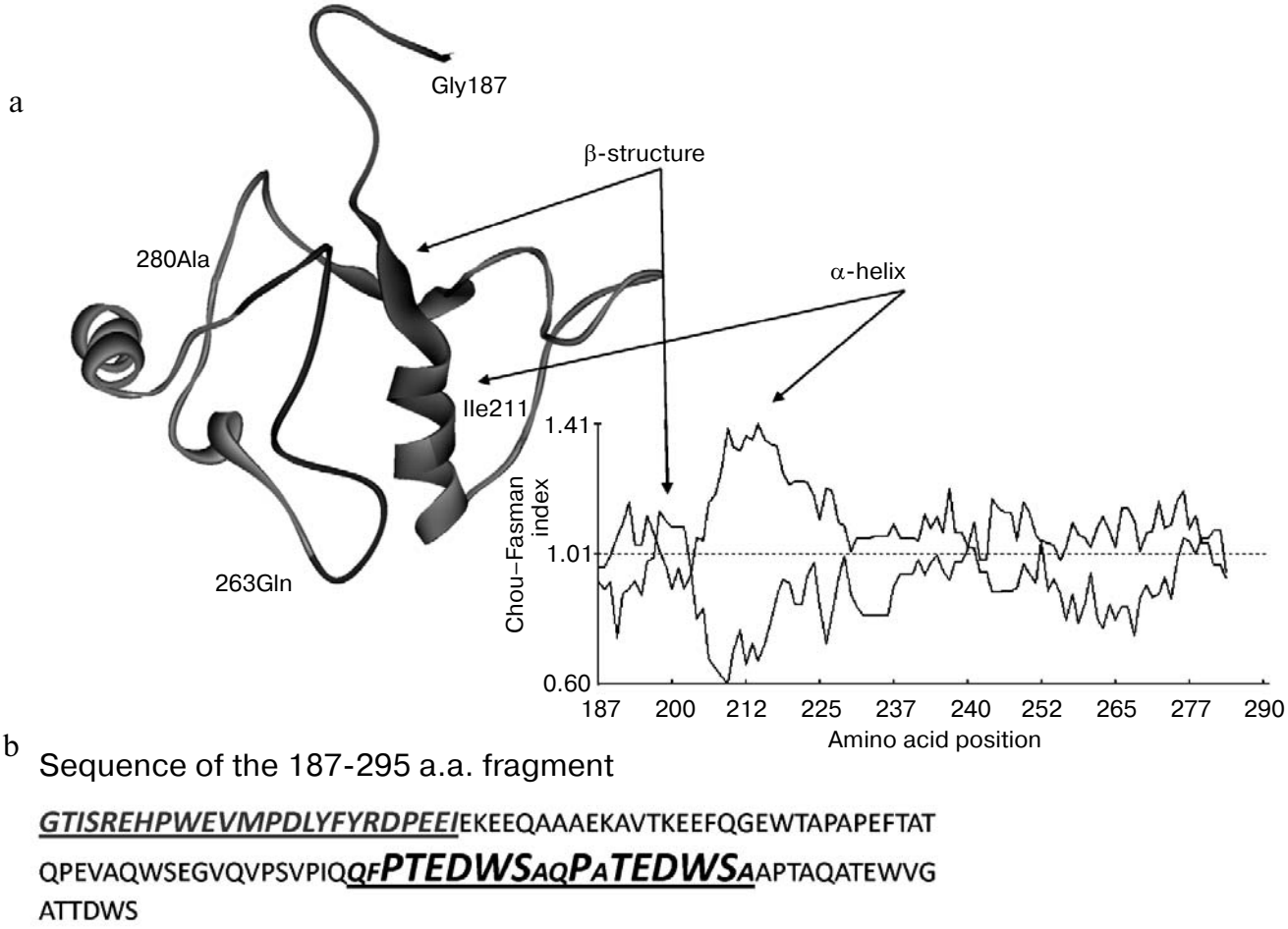

Fig. 4. Hypothetical model of spatial structure of the receptor domain of LBP. a) Spatial model of LBP receptor domain. b) Preference profile of amino acids by the formation of α- and β-structures (according to Chou–Fasman). Putative α- and β-structures are shown in gray, and mapping sequences are shown in italics.

laminin molecule. The second region is associated with amino acid residues 205-229 participating in direct interaction of LBP with laminin. According to the XRDA data, the region of residues 168-186 of LBP is represented by an α-helix followed by the surface loop of residues from 187 to 199. The location of the surface loop of residues 187-199 on the protein surface quite admits the possibility of representation of this region as a linear antigenic determinant for the interaction with MAB 8E4. Unfortunately, the region of residues 206-220 in the polypeptide used for XRDA had an indefinite structure, which prevented us from determining the spatial structure of this fragment of the C-terminal region of LBP. Previously, modeling of this region showed that it should contain an α-helical site [45]. Hydrophobic character of one of the sides of this α-helical region could provide for the interaction of LBP with heparin, and the second side of this region – the interaction with peptide 11 (CDPGYIGSR) of a laminin molecule.

The proposed epitope for MAB $4F6_{263\text{-}278}$ located at the C-terminal site of the LBP molecule contains a TEDWS peptide repeat. The functional activity of this peptide is not quite clear. Previously, for peptide QPATEDWSA it was shown that it could inhibit attachment of cancer cells to laminin-1 [45]. This suggested the participation of the above region of LBP in the interaction between LBP and a laminin molecule.

Recently, scFv recombinant antibodies to LBP have been obtained [16]. These antibodies were capable of reacting with the region 272-280 of LBP containing pentapeptide TEDWS and blocked the interaction between LBP and PrP prion protein *in vitro*. On passive administration to mice, S18 antibodies caused a 40% decrease in accumulation of PrP^{Sc} prion protein in the infected animal. This allowed the authors to suppose that scFv S18 antibodies to amino acid residues 272-280 of LBP could be a promising immunotherapeutic preparation for treatment of prion diseases. It is extremely important to note that MAB 4F6 blocking VEEV replication interacts with amino acid residues 263-278 of LBP, which is practically identical to the epitope for scFv S18 antibody (residues 272-280) possessing anti-prion activity. Practically com-

plete coincidence of epitopes for MABs 4F6 and scFv S18 reveals the involvement of this receptor domain of the LBP molecule containing a linear dimer of peptide TEDWS in the development of both viral and prion diseases.

Globular structure of the LBP molecule allows the spatial proximity of epitopes for MAB $8E4_{187\text{-}210}$ and MAB $4F6_{263\text{-}278}$ located far enough from each other in the amino acid sequence. These two MAB types partially compete with each other, which is indicative of the spatial proximity of binding epitopes for these antibodies. This suggests that the C-terminal fragment of LBP has the receptor domain (RD), which provides for the interaction of LBP with surface viral proteins of virions.

To test this supposition, we performed mathematical modeling of the spatial structure of LBP RD. The proposed model of the spatial structure of the LBP RD was distally limited by four linear loops, two of which were represented by experimentally mapped regions of residues 187-210 and 263-278, and the central β-folded and α-helical site including residues 200-216 of the LBP molecule. The proposed architectonics of the RD satisfactorily explains the properties of AIA to MABs 8E4 and 4F6 and their ability to interact with VEEV E2 protein and TBEV E protein. The proposed model suggests that the spatial structure of this region can have certain flexibility. This fact is indirectly confirmed by failures to obtain crystals of a full-length LBP molecule [9, 30]. Most probably, this fact will hamper further study of this region of the molecule with XRDA. The question of the role of two other linear loops of the RD and functional significance of individual amino acid residues in the formation of the ligand–receptor complex also remains open.

The importance and location of the RD at the C-terminal part of the LBP molecule confirm the previously obtained data of the study of MAB 1C3, which can block binding of Sindbis virus with cells [23]. MAB 1C3 interacted with a short fragment of the LBP molecule (residues from 248 to 295). The presence of competition for binding with LBP on the surface of mosquito cells C6/36 between VEEV and Sindbis virus provides additional evidence that these alphaviruses use the same receptor region. At the same time, the addition of laminin-1 to cells did not inhibit VEEV binding to the cells [19]. This is confirmed by the fact that alphaviruses and laminin-1 interact with different regions of LBP.

The conservative character of the spatial structure of LBP RD is confirmed by data on the interaction of LBP with E protein of West Nile virus and dengue virus. The pooled known data indicate that at least three flaviviruses (dengue, West Nile, and TBEV) and two alphaviruses (VEEV and Sindbis virus) use LBP to penetrate into sensitive cells [18-21]. Atomic-force microscopy was employed to determine the force of this protein–protein interaction; it was approximately 105 pN [22]. This suggests a certain universal and very specific mechanism for penetration of virions into the cell through the interaction of viral glycoproteins and an LBP molecule on the cell surface.

The efficiency of blocking of replication of some viruses and prions by antibodies to this receptor region of LBP demonstrates the prospects of further study of the mechanism for penetration of viruses into the cell through the interaction with LBP on the cell surface. High specificity of the virion–LBP interaction opens new opportunities for creation of antiviral preparations directionally blocking this receptor interaction. Possible efficacy of these preparations is confirmed by high activity of antibodies to LBP RD with respect to the development of viral infection on cell culture. The use of antibodies to the receptor domain of LBP for immunotherapy of viral and, probably, prion infections can become the next stage of development of this research.

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