Scheele, S. and Sasaki, T. and Arnal-Estape, A. and Durbeej, M. and Ekblom, P.

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Matrix Biology, 2006, Issue: April 19.

http://dx.doi.org/10.1016/j.matbio.2006.03.004

Monoclonal anti-mouse laminin antibodies: AL-1 reacts with laminin α1 chain, AL-2 with laminin β1 chain, and AL-4 with the coiled-coil domain of laminin β1 chain

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S.S. dedicates this paper to the memory of Peter Ekblom.

Abstract

We analyzed the reactivity of three different commercially available rat monoclonal antibodies raised against mouse laminin-$\alpha1\beta1\gamma1$ (laminin-111), AL-1, AL-2, and AL-4. Using ELISA assays, Western blot analysis and immunostainings we present refined epitope maps for these three laminin monoclonals. AL-1 reacted, as predicted with laminin $\alpha1$ chain. AL-4 has also been marketed as an $\alpha1$ chain specific probe, but we show here that AL-4 detects mouse laminin $\beta1$ chain, in the distal part of the coiled-coil region. AL-2 was predicted to react with all three chains near the cross region, but seems to primarily react with laminin $\beta1$ chain.

Keywords: Laminin, ELISA, monoclonal antibodies, recombinant proteins, kidney, muscle

Introduction

To date, 15 laminins have been described in mouse and human. They are large cross- or T-shaped heterotrimers consisting of different combinations of the currently known five α, three β, and three γ chains. The α1 chain is part of two known trimers, $\alpha1\beta1\gamma1$ and $\alpha1\beta2\gamma1$, named laminin (LM)-111 and LM-121. The laminin cross shape is achieved by a coiled-coil of the C-terminal regions of all three chains forming the long arm and each N-terminal forming the three short arms. In addition, the α chain alone forms the most distal part of the long arm due to its C-terminus consisting of five laminin globular (LG) domains (Aumailley et al., 2005).

Genetic evidence and culture of embryonic stem cells have established that laminin-111 is an essential morphogen for the first fetal differentiation in mice, starting when endoderm-derived LM-111 induces embryonic stem cell conversion to polarized epiblast cells (Smyth et al., 1999; Li et al., 2001; Li et al., 2002; Miner et al., 2004, Schéele et al., 2005). The α1 chain has also been implicated in kidney stem cell conversion into epithelial cells (Klein et al., 1988), lung development (Schuger et al., 1997), and mammary epithelial cell polarization (Gudjonsson et al., 2002). This is in line with evidence that α1 chain expression is largely limited to epithelial cells in embryos as well as to some epithelia in adult mice (Falk et al., 1999). The LM β2 chain is rarely coexpressed with the LM α1 chain which makes expression of LM-121 even more restricted. It has so far been reported in human placenta (Champilaud et al., 2000) and in muscle of laminin α2 chain deficient mice overexpressing LM α1 chain (Gawlik et al., 2004).

The restricted expression of the LM α1 chain is contrasted by the rather ubiquitously expressed LM β1 and γ1 chains as well as by the widely expressed β2 chain. In order to study LM-111 or LM-121 it is therefore essential to use antibodies specific to the LM α1 chain. Rat monoclonal antibodies AL-1 and AL-4 have, based on rotary shadowing, been suggested to detect α1 chain (Schuger et al., 1991). AL-1 was predicted to detect a central part of the LM-111 trimer containing all three chains, whereas AL-4 was shown to detect the end of the long arm (Skubitz et al., 1988), which led Schuger et al 1991 to suggest that it reacts with the α1 chain. Although convincing, rotary shadowing data does not provide enough resolution to allow distinction of chain specificity, as only a broad area can be implicated. AL-1 and AL-4 are nevertheless commercially marketed as α1-specific probes. AL-2, a commercially available antibody from the same series, is stated to react with the cross region of LM-111 (Skubitz et al., 1988; Schuger et al., 1991).

Here we characterized the reactivity of AL-1, AL-2, and AL-4, by ELISA, Western blot analysis, and immunostaining of adult kidney and skeletal muscle.

RESULTS AND DISCUSSION

Binding abilities of monoclonal antibodies AL-1, AL-2 and AL-4 in ELISA assays

As an initial approach to map the epitopes, ELISA assays using native and recombinant protein as well as protein fragments were performed. AL-1 was predicted by rotary shadowing to react with the LM α1 chain on or near the intersection of the cross. In ELISA it bound with low affinity to EHS-laminin, recombinant LM-111 and recombinant LM-121. It did not, however, bind to the N-terminal or the C-terminal of the LM α1 chain (Fig. 1A). According to these results, the epitope for AL-1 could be within the LM α1 or γ1 chains.

AL-2 was predicted to bind all three chains of LM-111 in the coiled-coil region close to the cross. In the ELISA assay, AL-2 was shown to bind well to EHS-laminin as well as to recombinant LM-111 (Fig. 1B). Furthermore, AL-2 bound with low affinity to LM-211, -411 and -511. No binding of recombinant LM-121 could be detected. The results show that the epitope of AL-2 is situated on the LM β1 chain. The binding further seems to be improved by the presence of the LM α1 chain.

By rotary shadowing and electron microscopy, the AL-4 was predicted to bind the end of the long arm, probably to the C-terminal globular domains of the LM α1 chain (Schuger et al., 1991). ELISA of AL-4 showed strong binding of EHS laminin, recombinant LM-111 as well as of the E8 fragment. However, no binding was detected for recombinant LM-121, the N-terminal fragment of the LM α1 chain, the β1 C-terminal fragment named 25k, or the C-terminal fragment of the LM α1 chain (Fig. 1C). Hence, the epitope for AL-4 may be situated on the LM β1 chain, between positions 1540 and 1679 which is within the E8 fragment but upstreams of the 25kD LM β1 C-terminal fragment.

Immunoblotting of laminin chains using monoclonal antibodies AL-1, -2 and -4

In order to test the reactivity of AL-1, -2 and -4, immunoblotting was performed using EHS-laminin as well as recombinant laminins. AL-1 reacted with EHS laminin, recombinant LM-111 and recombinant LM-121, but not with LM-211 under non-reducing conditions. No band was detected under reducing conditions (Fig. 2A).

Under reducing conditions, AL-2 recognized the 200 kD β1/γ1 and the 400 kD α1 band of EHS-laminin. Curiously, it did not bind the 400 kD α1 band of recombinant LM-111, whereas it bound well to the 200 kD β1/γ1 band. Furthermore, it bound with low affinity to the β1/γ1 band of LM-411 and -511. Using non-reducing conditions, AL-2 bound well to EHS-laminin and recombinant LM-111 as well as to LM-411 and -511. No binding of the AL-2 to LM-121 could be detected, neither under reducing conditions, nor under non-reducing conditions (Fig. 2B). In protein blots, the AL-2 monoclonal antibody thus seems to react primarily with the Lm β1 chain but under certain conditions also with the LM α1 chain.

AL-4 recognized, under reducing conditions, both the 200 kD band and the 400 kD band of EHS-laminin. However, the affinity for the 400 kD band was very low. AL-4 binding to LM β1 and γ1 together gave a strong band, whereas no binding to LM γ1 could be detected (Fig. 2C). As in the ELISA study, the epitope for the AL-4 seemed to be located on the LM β1 chain. It did, however, also recognize the LM α1 chain in immunoblots.

Reactivity of the AL-4 with adult kidney

The expression pattern of LM α1 in adult kidney is well established (Sorokin et al., 1997), and is largely confined to proximal tubules. This is in contrast to LM β1 and γ1 chains that are found in most basement membranes in the kidney. Using cryosectioned adult kidney, we performed immonofluorescent stainings with the monoclonal antibody AL-4. Based on the results from Western blot analysis and ELISA, AL-4 was predicted to react with LM β1 chain. Hence, counterstainings with antibodies directed against the LM α1 chain were performed. A composition image of the renal cortex and outer medulla shows that rabbit antisera against domain VI/V of the LM α1 chain N-terminal typically stained tubular basement membranes in the cortex as well as the inner part of the outer medulla (Sorokin et al., 1997). AL-4, however, stained all basement membranes, including those in the outer part of the outer medulla. The orientation is indicated in an adjacent section stained with hematoxylin/eosin. It was further shown that AL-4 reacts with the basement membranes in adipose tissue adjacent to the kidney. As expected, the antibody towards the LM α1 LG1-3 did not bind to the adipose basement membranes, but to a restricted set of tubular basement membranes in the kidney (Fig. 2D).

Reactivity of the AL-1, AL-2 and AL-4 with muscle

In contrast to the LM β1 and γ1 chains, the LM α1 chain is normally not detected in basement membranes associated with adult skeletal muscle. This tissue may thus serve as a negative control for antibody reactivity against the LM α1 chain. We performed immunofluorescent stainings, using AL-1, AL-2 and AL-4, of quadriceps from two week old wild type and LM α1 overexpressing transgenic mice. As controls we used a well characterized antibody against LM α1 chain, mab 200 (Sorokin et al., 1992) and an antibody against collagen IV. The latter should stain all basement membranes. Whereas the antibody against collagen IV stained both wild type and transgenic muscle, the mab 200, as expected, only stained transgenic muscle. The AL-1 showed the same staining pattern as the mab 200, but just as in the ELISA assay it reacted with low affinity. Most likely this is due to that AL-1 is commercially available only as ascites fluid. The AL-2 reacted with both wild type and transgenic muscle, although the affinity for the transgenic muscle appears to be slightly higher. Together with the results from Western blot analysis and ELISA, our findings suggest that AL-2 binds LM β1 and that the affinity is increased by the presence of the LM α1 chain. In line with the results from western blot and ELISA assays, AL-4 shows high reactivity with wild type as well as transgenic muscle (Fig. 2F).

In conclusion, the detailed mapping revealed that only AL-1 can be used to detect LM α1 chain, whereas the AL-2 and the AL-4 antibodies are specific for the LM β1 chain.

MATERIALS AND METHODS

ELISA and Western blot analysis

Purification of LM-111 from the mouse Engelbreth-Holm-Swarm tumour was carried out as previously described (Paulsson et al., 1987). The procedure for expression and purification of recombinant proteins was previously described (Mascarenhas et al., 2005). ELISA and Western blot analysis were carried out using standard procedures.

Immunofluorescence

Quadriceps muscle from wild-type and LM $\alpha 1$ overexpressing transgenic mice (Gawlik et al. 2004) and kidneys from wild-type mice were frozen in Tissue Tec and cryosectioned prior to immunofluorescent stainings. The following primary antibodies were used: monoclonal antibodies AL-1, AL-2 and AL-4 (Chemicon International), monoclonal antibody 200 (Sorokin et al. 1992), rabbit antisera against domain VI/V of the LM $\alpha 1$ N-terminus (Ettner et al 1998) and LM $\alpha 1$ LG 1-3 (Wizemann and Timpl, unpublished data) as well as a polyclonal antibody against collagen IV (Chemicon International). Secondary antibodies were Alexa 488 and Alexa 543 (Molecular probes). Images were captured with a Zeiss axioplan or a Leica confocal microscope and analysed with Adobe Photoshop software.

Acknowledgements

Supported by grants from Cancerfonden, Vetenskapsrådet (Stockholm), NovoNordisk (Copenhagen), and Deutsche Forschungsgemeinschaft (Bonn).

REFERENCES

Aumailley, M., Bruckner-Tuderman, L., Carter, W.G., Deutzmann, R., Edgar, D., Ekblom, P., Engel, J., Engvall, E., Hohenester, E., Jones, J.C.R., Kleinman, H., Martin, G.R., Mayer, U., Meneguzzi, G., Miner, J., Patarroyo, M., Paulsson, M., Quaranta, V., Sasaki, Sekiguchi, K., Sorokin, L., Talts, J.F., Tryggvason, K., Uitto, J., Virtanen, I., Yamada, Y., Yurchenco, P.D., 2005. A simplified nomenclature for the laminins. Matrix Biol. 24, 326-332.

Champilaud, M. F., Virtanen, I., Tiger C. F., Korhonon, R., Burgeson, R., and Gullberg, G., 2000. Posttranslational modification and beta/gamma chain association of human laminin α1 and laminin α5 chains: purification of laminin-3 from placenta. Exp Cell Res. 259, 326-35.

Ettner, N., Göhring W., Sasaki T., Mann K., Timpl R., 1998. The N-terminal globular domain of the laminin α1 chain binds to $\alpha1\beta1$ and $\alpha2\beta1$ integrins and to the heparan sulfate-containing domains of perlecan. FEBS lett. 430, 217-21.

Falk, M., Ferletta, M., Forsberg, E., Ekblom, P., 1999. Restricted distribution of laminin α1 chain in normal adult mouse tissues. Matrix Biol. 18, 557-568.

Gawlik, K., Miyagoe-Susuki, Y., Ekblom, P., Takeda, S., Durbeej, M., 2004. Laminin α1 chain reduces muscular dystrophy in laminin α2 chain deficient mice. Human Mol. Genet. 13, 1775-1784.

Gudjonsson, T., Ronnov-Jessen, L., Villadsen, R., Rank, F., Bissell, M.J., Petersen, O.W., 2002. Normal and tumor-derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. J. Cell Sci. 115, 39-50.

Klein, G., Langegger, M., Timpl, R., Ekblom, P., 1988. Role of laminin A chain in the development of epithelial cell polarity. Cell 55, 331-341.

Li, X., Chen, Y. Schéele, S., Arman, E., Haffner-Krausz, R., Ekblom, P., Lonai, P., 2001. FGF signalling and basement membrane assembly connected during epithelial morphogenesis of the embryoid body. J. Cell Biol. 153, 811-822.

Li, S., Harrison, D., Carbonetto, S., Fässler, R., Smyth, N., Edgar, D., Yurchenco, P.D., 2002. Matrix assembly, regulation, and survival functions of laminin and its receptors in embryonic stem cell differentiation. J. Cell Biol. 157, 1279-1290.

Mascarenhas, J. B., Rüegg, M. A., Sasaki, T., Eble, J. A., Engel, J. and Stetefeld, J. (2005) Structure and laminin-binding specificity of the NtA domain expressed in eukaryotic cells. Matrix Biol., 23, 507-513.

Miner, J.H., Li, C., Go, G., Sutherland, A.E., 2004. Compositional and structural requirements for laminin and basement membranes during mouse embryo implantation and gastrulation. Development 131, 2247-2256.

Paulsson, M., Aumailley, M., Deutzmann, R., Timpl, R., Beck, K. and Engel, J., 1987 Laminin-nidogen complex. Extraction with chelating agents and structural characterization. Eur. J. Biochem., 166, 11-19.

Schéele, S., Falk, M., Franzén, A., Ferletta, M., Ellin, F., Andersson, B., Timpl, R., Forsberg, E., Ekblom, P., 2005. Laminin α1 globular domains 4-5 induce fetal development, but are not involved in embryonic basement membrane assembly. Proc. Natl. Acad. Sci. USA 102, 1502-1506.

Schuger, L., Skubitz, A.P., O'Shea, K.S., Chang, J.F., Varani, J., 1991. Identification of laminin domains involved in branching morphogenesis: effects of anti-laminin monoclonal antibodies on mouse embryonic lung development. Dev. Biol. 146, 531-541.

Schuger, L., Skubitz, A.P., Zhang, J., Sorokin, L., He, L., 1997. Laminin α1 chain synthesis in the mouse developing lung: requirement for epithelial-mesenchymal contact and possible role in bronchial smooth muscle development. J, Cell Biol, 139, 5535-5562.

Skubitz, A.P., McCarthy, J.B., Charonis, A.S., Furcht, L.T., 1988. Localization of three distinct heparin-binding domains of laminin by monoclonal antibodies. J. Biol. Chem. 263, 4861-4868.

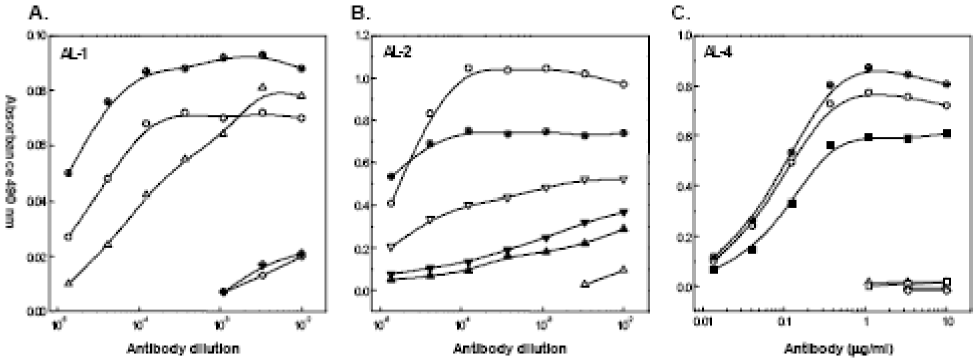
Smyth, N., Vatansever, H.S., Murray, P., Meyer, M., Frie, C., Paulsson, M., Edgar, D., 1999. Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. J. Cell Biol. 144, 151-160.

Sorokin L., Conzelmann S., Ekblom P., Battaglia C., Aumailley M., Timpl, R., 1992. Monoclonal antibodies against laminin A chain fragment E3 and their effects on binding to cells and proteoglycan and on kidney development. *Exp. Cell Res.* 201, 137-144.

Sorokin, L., Pausch, F., Durbee,j M., Ekblom, P., 1997. Differential expression of five laminin α chains (1-5) in developing and adult mouse kidney. Dev. Dyn. 210, 446-462.

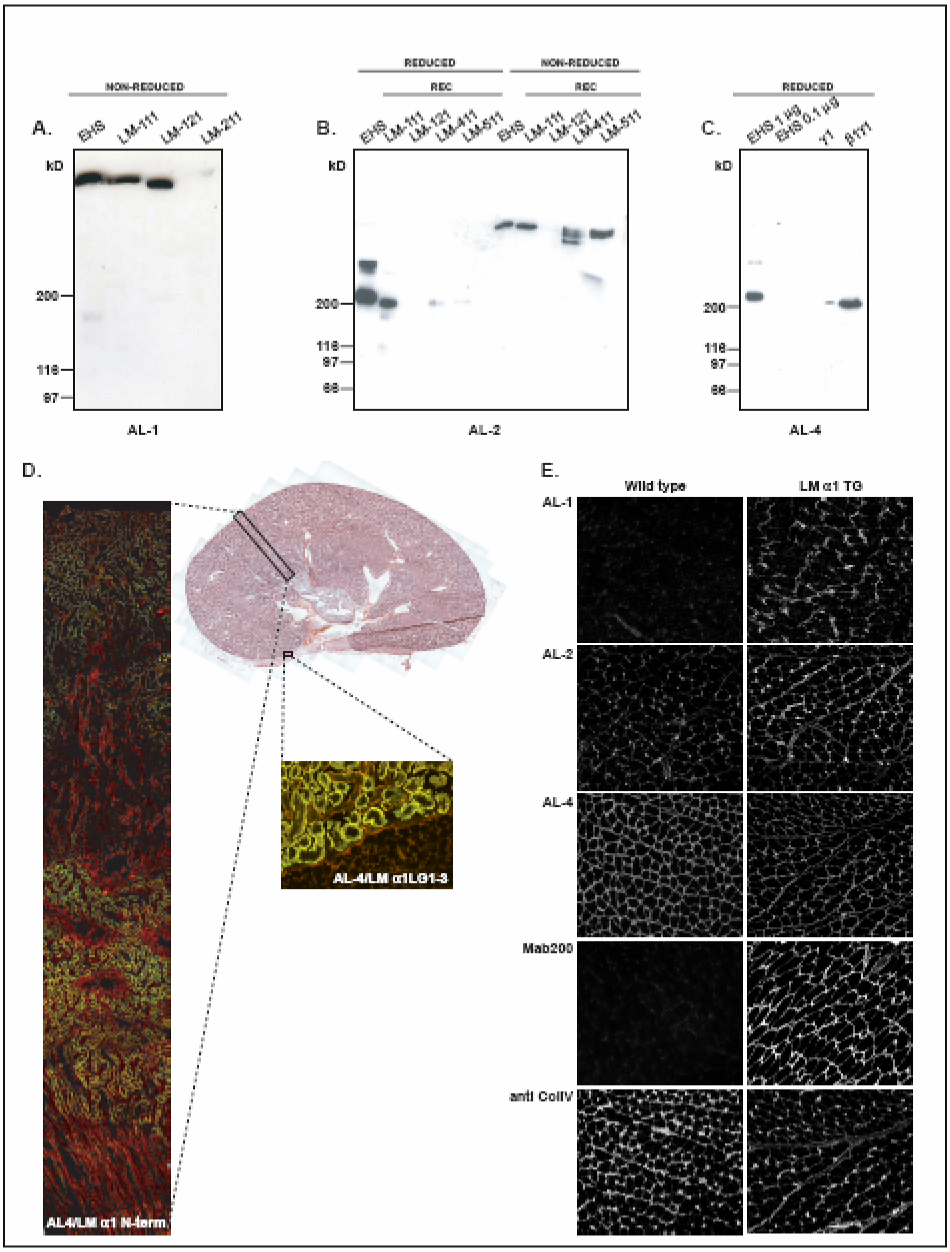
Figure legends.

Figure 1.



ELISA titration of AL-1(A), AL-2(B) and AL-4(C). Antigens used were EHS-laminin (○), recombinant laminin-111(●), recombinant laminin-121(△), recombinant laminin-211(▲), recombinant laminin-411(▽), recombinant laminin-511(▼), N-terminal fragment of LM α1 chain [residues 25-2020] (◇), C-terminal fragment of LM α1 chain [residues 2021-3084](◆), E8 fragment (■) and C-terminal short coiled-coil fragment of LM β1 chain [residues 1748-1834](□).

Figure 2.



(A, B, C) Reactivity of AL-1, AL-2 and AL-4 in immunoblots. In A, 1µg of each laminin was loaded. In B, for EHS laminin and recombinant LM-111, 1µg of each laminin was used under reducing condition and 0.2 µg of each were used under non-reducing condition. For recombinant LM-411 and LM-511, the same amounts were separated under both conditions. In C, EHS laminin, the cell lysate from LM γ1 chain-transfected cells (γ1) and from LM β1 and γ1 chains-transfected cells (β1γ1) were loaded under reducing condition.

(D) Staining of the AL-4 in adult kidney. Staining with the AL-4 antibody (red) and with rabbit antisera against domain VI/V of the LM α1 N-terminus (green) co-localize (yellow) in the outer cortex and the outer medulla of adult kidney, but not in the inner part of the cortex. The AL-4 antibody (red) stained the basement membranes of kidney as well as of adipose tissue, whereas staining of LM α1 LG1-3 could be detected in some kidney basement membranes but not in adipose tissue.

(E) Immunostainings of wild type and transgenic quadriceps muscle. As expected of an antibody directed towards LM α1 chain, the AL-1 did not stain wild-type quadriceps, but showed a weak staining of the transgenic LM α1 chain over expressing quadriceps. The same pattern can be observed with the mab200 antibody that binds to the LG4 of the LM α1 chain. AL-2 binds to both wild type and transgenic quadriceps, although the affinity for the transgenic muscle seems slightly higher. AL-4 binds well both to wild type and transgenic muscle as do the antibody against collagen IV.