Characterization of the Laminin Binding Domains of the Lutheran Blood Group Glycoprotein*

Lutheran (Lu) blood group antigens and the basal cell adhesion molecule antigen reside on two glycoproteins that belong to the Ig superfamily (IgSF) and carry five Ig-like extracellular domains. These glycoproteins act as widely expressed adhesion molecules and represent the unique receptors for laminin-10/11 in erythroid cells. Here, we report the mapping of IgSF domains responsible for binding to laminin. In plasmonic resonance surface experiments, only recombinant Lu proteins containing the N-terminal IgSF domains 1–3 were able to bind laminin-10/11 and to inhibit binding of laminin to Lu-expressing K562 cells. Mutant recombinant proteins containing only IgSF domain 1, domains 1 + 2, domains 1 + 3, domains 2 + 3, domain 3, domain 4, domain 5, and domains 4 + 5 failed to bind laminin as well as a construct containing all of the extracellular domains except domain 3. Altogether, these results indicate that IgSF domains 1–3 are involved in laminin binding and that a specific spatial arrangement of these three first domains is most probably necessary for interaction. Neither the RGD nor the N-glycosylation motifs present in IgSF domain 3 were involved in laminin binding.


during erythroid differentiation (14, 15) and represent the unique receptors of laminin in normal and sickle red blood cells as well as in erythroid progenitors (9, 10). Increased expression of Lu antigens on sickle red blood cells correlates with an increased adhesion to laminin (9, 10), which, together with other adhesion molecules like CD36 and VLA-4, might contribute to the reinforced adhesion of the sickle red blood cells to vascular endothelium leading to vascular occlusion crisis and strokes (16, 17).

The use of domain deletion mutants provided the first information on the structure/function relationship by showing that at least one Lu polymorphic blood group antigen is located on each of the five IgSF domains (18). In addition, the membrane-proximal IgSF domain 5 was shown to be the only critical domain for laminin binding (19). In the present study, we further extended these studies by generating deletion mutants of the NH2 extracellular domain of Lu gps and mutants of the RGD and glycosylation motifs in order to better characterize the binding site and protein motifs involved in the interaction with human laminin. By the use of direct interaction biosensor assays and inhibition assays with a flow cytometer, we found that the first three N-terminal Ig-like domains are critical for interaction with laminin. Moreover, neither the RGD nor the N-glycosylation motifs of the third IgSF domain are involved in this interaction.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfection, and Flow Cytometry Analysis—Human K562 cells were obtained from the American Type Culture Collection.

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Wassim El Nemer‡, Pierre Gane‡, Yves Colin‡, Anne Marie D’Ambrosio‡, Isabelle Callebaut‡, Jean-Pierre Cartron‡, and Caroline Le Van Kim‡

From the ‡INSERM U76, Institut National de la Transfusion Sanguine, Paris 75015, France and §CNRS UMR7590, Universités Paris 6 et Paris 7, Paris 75005, France

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To whom correspondence should be addressed: INSERM U76, Institut National de la Transfusion Sanguine, 6 rue Alexandre Cabanel, 75015 Paris, France. Tel.: 33 1 44 49 30 46; Fax: 33 1 43 06 50 19; E-mail: levankim@idf.inserm.fr.

The abbreviations used are: Lu, Lutheran; gp, glycoprotein; IgSF, immunoglobulin superfamily; RU, response units; ICAM, intercellular adhesion molecule.
(Manassas, VA) and were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Stable transfected K562 cells expressing Luβ isoform were obtained as described (10). Expression of Luβ antigen on transfectant cell lines was measured on a FACScan flow cytometer (Becton Dickinson, San Jose, CA) using LM342 monoclonal antibody (gift of Dr. Colin Fraser, Regional Donor Center, Glasgow, UK) as described (20).

**Production of Soluble Recombinant Lu-Fc—**Recombinant cDNAs encoding different extracellular domains of the Lu gp were obtained by polymerase chain reaction using the CDNA GC-rich kit (CLONTECH) and the Luα CDNA in pcDNA3 vector as template (10). The polymerase chain reaction products were subcloned into the pGEM vector (Promega). Expression of the Lu1-4-Fc was performed in COS cells (Amersham Pharmacia Biotech), and the Lu-Fc proteins were eluted as recommended by the manufacturer. Recombinant Fc protein concentrations were assessed by colorimetric assay using a Bio-Rad protein assay kit, and the integrity of the preparations was checked by 8% SDS-polyacrylamide gel electrophoresis and staining with silver nitrate.

**Laminin Binding Assay—**Biosensor studies were performed on a Biacore X instrument (Amersham Pharmacia Biotech). All experiments were performed at 25 °C using an HBS buffer, comprising 10 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM EDTA. Rabbit anti-human Fc (Pierce) was diluted in a 10 mM acetate buffer, pH 4.5, and covalently coupled to a CM-5 or to an F1 biosensor chip by amine coupling, using an amino coupling kit (Amersham Pharmacia Biotech). Lu-Fc and Fc control proteins were diluted in HBS buffer and coupled to the anti-human Fc antibody. Typically, a coupling level of 200–400 and 10–20 response units (RU) were used for mapping and kinetic analyses, respectively. Different concentrations of immunopurified laminin-10/11 (Life Technologies, Inc.) or control fibronectin and merosin (laminin-2) proteins were injected over the coupled Lu-Fc surface as well as a control protein surface (ICAM-4-Fc) at a flow rate of 10 μl/min. A 25 mM NaOH solution was used for regeneration of the immobilized surface between different Lu-Fc injections. Sensorgrams obtained for the Fc control protein were systematically subtracted from those obtained for Lu-Fc proteins. For kinetic analysis, a range of six different laminin concentrations were injected (5, 8, 10, 16, 20, and 30 nM) at a flow rate of 30 μl/min with an R_{max} not exceeding 100 RU. Affinity constants were estimated by curve fitting using BIA Evaluation 3.0 software assuming a 1:1 binding model.

In another series of experiments, laminin-2 and laminin-10/11 were diluted in 10 mM acetate buffer, pH 3.3, and covalently coupled (9,000–10,000 RU) to flow cells FC1 and FC2, respectively, of a CM-5 biosensor chip by amine coupling using an amino coupling kit (Amersham Pharmacia Biotech). Lu-Fc and Fc control proteins were diluted in the HBS buffer and injected over the laminin covered surface at a flow rate of 10 μl/min. Sensorgrams obtained for laminin-10/11 were subtracted from those obtained for laminin-2.

**Laminin Binding Inhibition Assay—**Laminin binding inhibition assay using secreted chimeric Lu-Fc fragment was performed as follows. 500-ng aliquots of laminin were incubated with different dilutions of the Lu-Fc fragment or a control Fc fragment for 1 h at 37 °C in PBS, supplemented with 0.5% bovine serum albumin. The suspensions were then added to 5 × 10^5 K562 cells expressing the Luβ isoform and incubated for 45 min at room temperature. After two washes with PBS, cells were incubated with the anti-laminin monoclonal antibody (anti-A chain) (Roche Molecular Biochemicals) for 30 min at 4 °C. The cells were then washed twice with PBS and incubated with phycoerythrin-conjugated anti-mouse IgG (ImmunoTech, Marseille, France) for 20 min at 4 °C. After another washing step, 0.1 μg of TO-PRO-1 iodide (Molecular Probes, Inc., Eugene, OR) was added, and positive cells (dead cells) were excluded from analysis.

**Sequence Analysis, Secondary Structure Predictions, and Homology Modeling—**Sequences of the five IgSF domains were searched against the nonredundant database (21) for homology against IgSF and C2-set profiles. The Protein Data Bank was also searched using PSI-BLAST (22) and FUGUE2 for predicting the Lu domain’s secondary structures relative to experimentally solved structures of Ig-like domains. Homology modeling of Lu IgSF domain 3 was performed using J. Shi, T. L. Blundell, and K. Mizuguchi, manuscript in preparation.

**RESULTS AND DISCUSSION**

**Interaction of the Recombinant Lu12345-Fc Fusion Protein with Laminin-10/11—**The interaction of the common extracellular region of Lu and Lu(v13) gps with laminin was first analyzed by plasmon resonance experiments using a Biacore X instrument. This region of 518 amino acids is organized in five IgSF domains, noted Lu12345 thereafter. The sequence coding for Lu12345 was fused to that coding for the Fc fragment of a human IgG, and the soluble chimeric Lu-Fc fusion protein was expressed in COS-7 cells (see "Experimental Procedures"). After purification on Protein A-Sepharose, the chimeric polypeptide migrated as an expected single band of 106 kDa (Fig. 1). For biosensor assays, the rabbit anti-human Fc antibody was immobilized directly to a carboxylated dextran matrix chip, and the Lu12345-Fc fusion protein was captured on the matrix and tested for interaction with laminin. As shown in Fig. 2, protein laminin 10/11 bound to Lu12345-Fc in a dose-responsive fashion in the range of 5–30 nM laminin. The specificity of the binding interaction was determined by showing that laminin did not bind to the immobilized adhesion molecule ICAM-4-Fc, which contains two IgSF domains (23, 25). In further control experiments, it was found that neither laminin-2 (α2β1γ1) nor fibronectin at 20 nM bind to either immobilized Lu12345-Fc or ICAM-4-Fc fusion proteins (data not shown). For kinetic analysis, small amounts of Lu12345-Fc were captured on the anti-human Fc surface in order not to exceed an R_{max} of 50–100 RU when injecting saturating concentrations of laminin. After injecting a range of six different concentrations (5, 8, 10, 16, 20, and 30 nM), kinetic analysis revealed that Lu12345-Fc bound laminin with high affinity, with a dissociation equilibrium constant K_d of 10.8 nM (kinetic constant k_{a} = 2.45 × 10^6 M^{-1} s^{-1}). This value correlates well with recently reported independent studies (6). Recent studies indicated that commercial human laminin preparations contain laminin 10 (α2β1γ1) and some laminin 11 (α5β2γ1) but no laminin 1 (26). Since α5 chain is present only in...
laminin 10 and 11 isoforms (27) and Lu gps bind neither to murine laminin 1 ($a_1b_1g_1$) nor to laminin 2 ($a_2b_1g_1$) (9, 10, 28), it was postulated that Lu gps bind to laminin-10/11 via the specific $a_5$ chain (Ref. 6 and this report), which is also in agreement with other studies showing that sickle red blood cells adhere to laminin-10/11 isoforms (29). Furthermore, the laminin $a_5$ chain is expressed in bone marrow and by several developing epithelial cells, and a majority of epithelial cells might use laminin-10 rather than laminin-1 as an adhesive protein in vivo (26, 30).

Interaction between Lu gp Mutants and Laminin-10/11—In order to identify the IgSF domain(s) that interact(s) with laminin-10/11, 11 IgSF domain deletions (three small deletions of five amino acids and two single amino acid mutants) were generated and expressed as Fc fusion proteins. The schematic structure of these mutants is shown on Fig. 3. To generate entire domain removal, deletion sites were chosen in the interdomain sequences deduced from the putative structure of the Lu gp (1). The Lu12345-Fc protein containing the five extracellular IgSF domains was used as a positive control when analyzing the interaction between the different mutated proteins and laminin-10/11. The four deletion constructs Lu123-Fc, Lu12-Fc, and Lu1-Fc were first analyzed by SDS-PAGE (Fig. 1), and among these, we found that only Lu123-Fc and Lu1234-Fc bound to laminin (Fig. 4). The lack of interaction between laminin and Lu1-Fc and Lu12-Fc proteins was not due to defective chimeras, since these proteins were able to bind to monoclonal antibody LM342, which recognizes the Lub epitope localized in the first IgSF domain in Biacore experiments (data not shown). Kinetic analysis also revealed that Lu123-Fc and Lu12345-Fc had similar binding affinity for laminin 10/11 ($K_d = 9.8 \text{nM}$ versus 10.8 nM), and this result, together with those reported above, suggests that the laminin binding site(s) of Lu gps includes the third IgSF domain and that domains 4 and 5 neither enhance nor decrease laminin binding affinity. Accordingly, deletion mutants Lu4-Fc, Lu5-Fc, and Lu45-Fc did not bind to laminin 10/11 (Fig. 3 and 4).

These results are in general agreement with a recent study published when this work was being written (6). However, our studies further extended this analysis, since a panel of nine other Lu chimeras carrying different Ig domain deletions, small deletions, and single point mutations into IgSF domain 3 (see Fig. 3) were investigated.

The laminin binding capacity of Lu123-Fc but not of Lu12-Fc constructs pointed to the important role of domain 3 of Lu gp for ligand binding. To confirm this result, we first generated a mutant protein lacking domain 3 only. As shown in Fig. 4A, this Lu1245-Fc chimera did not bind to laminin-10/11. To test whether the N-glycan of domain 3 is important for laminin binding, asparagine 321 was mutated into alanine to abolish the unique potential N-glycosylation site of the first three IgSF
domains, which is well exposed at the beginning of strand E according to the structural model proposed in Fig. 5B. The LuN321A-Fc construct directed the synthesis of a soluble protein with an apparent molecular mass of 103 kDa versus 106 kDa for the wild type protein (Fig. 1). This indicates that the Asn321 glycosylation site is used, and the loss of about 3 kDa is compatible with the removal of a single N-glycan chain. Arginine 292 belonging to a potential RGD integrin binding site of domain 3 was also mutated into alanine (construct LuR292A-Fc), since the RGD motif is commonly involved in cell adhesion and is crucial for some integrin binding and for the binding of many proteins to cells, such as fibronectin and transferrin to their cognate receptors (31, 32). As shown in Fig. 4B, the sensogram of the interaction between laminin-10/11 and LuN321A-Fc and LuR292A-Fc constructs is similar to that obtained with Lu12345-Fc, indicating that neither N-glycosylation nor the RGD motif of domain 3 were involved in the interaction with laminin 10/11. This conclusion is also supported by the finding that the mouse homologue of Lu gp binds human laminin but does not contain any RGD motif (6, 28).

Moreover, as shown on the model of the Lu IgSF domain 3 structure (Fig. 5B), the RGD sequence is not included in a loop, as observed for fibronectin RGD sequence (33) but lies extended in the C-terminal part of strand βB, just after the cysteine residue implicated in disulfide bonding with the strand βF cysteine, and therefore has a different presentation as compared with canonical RGD.

Next, three different small deletions of five amino acids were introduced into the third IgSF domain of the Lu12345-Fc protein (see Fig. 3). All deleted amino acids are conserved between human, mouse, and rat (Fig. 5A). These are predicted to be localized in three regions included in distinct secondary structures, namely the B-C loop and the beginning of the C strand (Lu ΔPSPEY-Fc), the F strand (Lu ΔRVEDY-Fc), and the D strand and the beginning of the D-E loop (LuΔLNVLN-Fc) (Fig. 5B). As shown in Fig. 4B, these small deletions within domain 3 drastically alter the association and dissociation curves and reduce the laminin binding property of the extracellular region of the Lu gps. Disruptions of regular secondary structures in which these segments participate and the nonobservance of geometrical constraints probably impede the correct folding of domain 3. It is also worth noting that two of the deleted segments (ΔPSPEY-Fc and ΔRVEDY-Fc) contain two amino acids (underlined Y and V, respectively) whose positions are always occupied by hydrophobic amino acids in the multiple alignment of C2 domains and are buried within three-dimensional structures (data not shown). These amino acids are therefore predicted to be essential to the maintenance of the IgSF fold, and their absence would thus drastically impair the correct folding of domain 3. Although these results confirm the importance of the third domain, it could not be concluded whether domain 3 alone, albeit critical, was sufficient for laminin binding. Indeed,
nin-10/11 aliquots (500 ng) were preincubated with increasing amounts of the recombinant Lu-Fc proteins (0.625, 2.5, and 10 pM) before the addition to K562 cells expressing the Lu gp isofrom. The percentage of bound laminin is expressed as the relative fluorescence intensity versus Fc fragment concentration. Only chimeric proteins containing the first three IgSF domains (Lu12345-, Lu1234-, and Lu123-Fc) are inhibitors. Lu1- and Lu12-Fc do not inhibit laminin binding to K562.Lu cells.

It is often observed that the combination of several IgSF domains is necessary for interaction with partners, as discussed below. To further address this issue, a mutant protein containing only domain 3 (Lu3-Fc) was generated. As shown in Fig. 4A, Lu3-Fc did not bind to laminin-10/11 even when large amounts of laminin were injected, indicating that the third IgSF domain of Lu gp is necessary but not sufficient to mediate laminin-10/11 binding. Together with the positive binding of Lu123-Fc to laminin, these results indicated that domain 1 and/or 2 may also play a role in the interaction process. To find out whether the interaction involves domain 1 or 2, Lu13-Fc and Lu23-Fc chimeras were generated, but none of these two chimeras were found to bind to laminin-10/11 (Fig. 4A).

To confirm these results, we performed a reverse series of biosensor assays in which laminin-10/11 was covalently coupled to the sensor chip, and several dilutions of Lu-Fc chimeras were injected. Laminin-2 was used as a negative control. Lu12345-Fc, Lu1234-Fc, Lu123-Fc, LuN321A-Fc, and LuR292A-Fc showed typical specific binding curves with laminin-10/11, whereas the other Lu-Fc chimeras did not bind to the immobilized laminin (data not shown).

Inhibition of Lu-Laminin Binding by the Lu-Fc Soluble Proteins—Previous adhesion and flow cytometric analyses have shown that K562 cells expressing Lu gps bind laminin (9, 10) and that the Lu12345-Fc chimera protein doseresponsively inhibited the laminin-Lu gp interaction (10). To further confirm the BIAcore results (see above), we have used the different Lu-Fc chimeras to inhibit the interaction of laminin with K562.Lu cells. Increasing amounts of each recombinant protein were preincubated with 500 ng of laminin-10/11 prior to the binding assay with K562.Lu cells. Among all the mutated proteins, only Lu123-Fc, Lu1234-Fc, LuN321A-Fc, and LuR292A-Fc molecules dose-responsively inhibited the binding of laminin to K562.Lu cells, as did the control Lu12345-Fc protein (Fig. 6). More than 80% inhibition was obtained with 10 pM of these mutated constructs. The inhibition assays largely confirm the BIAcore results and indicated again that laminin binding to K562.Lu cells requires the three first Ig-like domains of Lu gp.

All these results suggested that the interaction between laminin-10/11 and Lu gps involves sequences present in the three extracellular domains 1, 2, and 3, which might be arranged together so that they provide a specific interaction surface. Lu gps share sequence similarities with cell adhesion molecules belonging to the IgSF (e.g. neural cell adhesion molecule, axonin, etc.), which are similarly organized (i.e. with an N-terminal string of IgSF domains (5 and 6 for neural cell adhesion molecule and axonin, respectively). Interestingly, the first four IgSF domains of axonin (this is also true for hemolin) adopt a U-shaped arrangement, forming a compact four-domain module with contacts between domains 1 and 4 and between domains 2 and 3 (34). By analogy, as the IgSF domain 4 of Lu gp does not appear critical for laminin binding (see Fig. 4A), it is suggested that the first three IgSF domains of Lu gp could constitute a three-domain module having a specific spatial arrangement with domains 1–3 playing a key role in the interaction properties. Similarly, the interaction between laminin and activated leukocyte cell adhesion molecule apparently requires the first two IgSF domains only (35).

Independently, two groups have reported the characterization of the laminin binding site on the fifth (19) or the first three (6) IgSF domains of the Lu gps. Here, we have shown by two different techniques, using purified chimeric proteins and Lu-expressing cells, that the N-terminal domains 1–3 are critical for laminin-10/11 interaction, since neither domain 1, domains 1 + 2, domain 3, domains 1 + 3, nor domains 2 + 3 were able to mediate laminin interaction. In addition, the present study demonstrates that the structural integrity of the IgSF domain 3 is necessary for laminin-10/11 interaction and that neither N-glycosylation nor RGD motifs of this domain were involved in laminin-10/11 binding. Our results and those recently reported (6) apparently conflict with those from Zen et al. (19) that have assigned the laminin binding domain to the fifth IgSF domain of Lu gp. Although different techniques of investigation have been used, and much fewer mutants analyzed compared with the present report, the reason for this discrepancy is not clear, but we cannot exclude the possibility that Lu gps may have two distinct binding sites for laminin.

Further investigation should document the critical residues of Lu gps involved in laminin interaction and reciprocally those involved in the laminin α5 chain counterpart more precisely. This information may be useful to develop inhibitors that block the Lu-laminin interaction and may reduce the increased adhesion of sickle red cells to laminin, which is suspected to contribute to vaso-occlusion in patients (16, 17). This will be of great importance also, since Lu antigens are overexpressed in some epithelial cancer tissues (36, 37) and since laminin is involved in cancer metastases (38).

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REFERENCES
1. Parsons, S. F., Mallinson, G., Holmes, C. H., Houlihan, J. M., Simpson, K. L., Mawby, W. J., Spurr, N. K., Warne, D. A., Bax, K. N., and Anstee, D. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5496–5500
2. Rahuel, C., Le Van Kim, C., Mattei, M. G., Cartron, J. P., and Colin, Y. (1996) Blood 88, 1865–1872
3. Yu, H., Chen, J. K., Feng, S., Dalgarno, D. C., Brauer, A. W., and Schreiber, S. L. (1994) Cell 76, 933–945
4. El Nemer, W., Colin, Y., Bassy, C., Codogno, P., Fraser, R. H., Cartron, J. P., and Le van Kim, C. (1999) J. Biol. Chem. 274, 31903–31908
5. Gane, P., Le Van Kim, C., Bony, V., El Nemer, W., Mourou, I., Nicolas, V., Colin, Y., and Cartron, J. P. (2001) Br. J. Haematol. 113, in press
6. Parsons, S. F., Lee, G., Spring, F. A., Willeit, T. N., Peters, L. L., Gimm, J. A., Tanner, M. A., Mohandas, N., Anstee, D., and Chasis, J. A. (2001) Blood 97, 312–320
7. Johnson, J. P., Rothbacher, U., and Sers, C. (1993) Melanoma Res. 3, 337–340
8. Bowen, M. A., Patel, D. D., Li, X., Modrell, B., Malacon, A. R., Wang, W. C.,
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9. Udani, M., Zen, Q., Cottman, M., Leonard, N., Jefferson, S., Daymont, C., Truskey, G., and Telen, J. P. (1998) J. Biol. Chem. 273, 16686–16693

10. Udani, M., Zen, Q., Cottman, M., Leonard, N., Jefferson, S., Daymont, C., Truskey, G., and Telen, J. P. (1998) J. Biol. Chem. 273, 16686–16693

11. Engel, J. (1992) Biochemistry 31, 10643–10651

12. Tryggvarson, K. (1993) Curr. Opin. Cell Biol. 5, 877–882

13. Yurchenko, P. D., O'Rear. (1993) in Molecular and Cellular Aspects of Basement Membranes (Rohrbach, D. H., Timpl, R., eds) pp. 121–146, Academic Press, Inc., San Diego, CA

14. Bony, V., Gane, P., Bailly, P., and Cartron, J. P. (1999) Br. J. Haematol. 107, 263–274

15. Southcott, M. J. G, Tanner, M. J. A., and Anstee, D. J. (1999) Blood 93, 4425–4435

16. Wick, T. W., and Eckman, J. R. (1996) Curr. Opin. Hematol. 3, 118–124

17. Hebbel, R. P. (1997) J. Clin. Invest. 101, 2550–2558

18. Parsons, S. F., Mallinson, G., Daniels, G. L., Green, C. A., Smythe, J. S., and Anstee, D. J. (1999) Blood 93, 4219–4225

19. Zen, Q., Cottman, M., Truskey, G., Fraser, R., and Telen, M. J. (1999) Blood 93, 4608–4616

20. El Nemer, W., Rahuel, C., Colin, Y., Goossens, D., Gane, P., El Nemer, W., Cartron, J. P., and Le Van Kim, C. (1999) Immunogenetics 50, 271–277

21. Schultz, J., Copley, R. R., Doerks, T., Ponting, C. P., and Bork, P. (2000) Nucleic Acids Res. 28, 231–234

22. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402

23. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815

24. Bailly, P., Tontti, E., Hermand, P., Carton, J. P., and Gahmberg, C. G. (1995) Eur. J. Immunol. 25, 3316–3329

25. Hermand, P., Hu, M., Callebaut, I., Gane, P., Ilhanus, E., Gahmberg, C. G., Cartron, J. P., and Bailly, P. (2000) J. Biol. Chem. 275, 26092–26010

26. Ferletta, M., and Ekblom, P. (1999) J. Cell Sci. 112, 1–10

27. Miner, J. H., Patton, B. L., Lentz, S. I., Gilbert, D. J., Sinder, W. D., Jenkins, N. A., Copeland, N. G., and Sanes, J. R. (1997) J. Cell Biol. 137, 685–701

28. Rahuel, C., Colin, Y., Goossens, D., Gane, P., El Nemer, W., Cartron, J. P., and Le Van Kim, C. (1999) Immunogenetics 50, 271–277

29. Lee, S. P., Cunningham, M. L., Hines, P. C., Joneckis, C. C., Orringer, E. P., and Parise, L. V. (1998) Blood 92, 2951–2958

30. Gu, Y., Sorokin, L., Durbeej, M., Bjork, T., Jonsson, J. I., and Ekblom, M. (1999) Blood 93, 2533–2542

31. Ruoslahti, E. (1996) Tumour Biol. 17, 117–124

32. Dubljevic, V., Sali, A., and Goding, J. W. (1999) Biochem. J. 341, 11–14

33. Ruoslahti, E. (1996) Annu. Rev. Cell Dev. Biol. 12, 697–715

34. Freigang, J., Proba, K., Leder, L., Diederichs, K., Sonderegger, P., and Welte, W. (2000) Cell 101, 425–433

35. Zen, Q., Riddle, M. E., Fong, A. M., Patel, D. D., Paulsen, D. M., Truskey, G., and Telen, J. P. (1999) FASEB J. 13, A1360 (Abstr. 171)

36. Chesa-Garin, P., Sanz-Moncasi, M. P., Campbell, I. G., and Rettig, W. J. (1994) Int. J. Oncol. 5, 1261–1266

37. Rettig, W. J., Gari-Chesa, P., Beresford, H. R., Oettgen, H. F., Melamed, M. R., and Old, L. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3110–3114

38. Terranova, V. P., Liotta, L. A., Rousso, R. G., and Martin, G. R. (1982) Cancer Res. 42, 2265–2269