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MULTIPLE LAMININ RECEPTORS — A RADIOLIGAND BINDING APPROACH

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Laminin (LN) is a large multifunctional glycoprotein, specific from basement membranes, involved in a series of biological phenomena, including cell adhesion, growth and differentiation. These functions are mediated by specific cell surface receptors, as discussed in detail in this issue (M. M. Brentani, 1991; R. Chammas et al., 1991).

In this report, we analyze a radioligand binding assay as a tool for characterization of specific LN receptors. The model studied is a murine melanoma cell line (M2R), derived from the classical B16 model (Mather & Sato, 1979, *Exp. Cell Res., 120*: 191).

These cells do not express endogenous LN, at least as determined by usual immunocytochemical analysis (immunofluorescence and immunoblot from cell surface extracts). Under culture conditions, there is no exogenous source of laminin; thus, in this model, putative LN receptors would be unoccupied and free to diffuse through the plasma membrane.

M2R cell membrane extracts were applied to a LN-Sepharose column and then sequentially eluted with a NaCl gradient (from 0.1 to 1.0 M, Fig. 1A) and a low pH solution (glycine 0.1 M, pH 2.0, Fig. 1B). Under these conditions, it was possible to elute four distinct LN binding proteins (82, 67, 28 and 120 kDa), characterizing the heterogeneity of the model studied, regarding LN receptors.

We standardized a method for evaluating LN binding parameters on cells adhered on plastic, under defined culture conditions. In these assays, M2R cells were incubated with increasing amounts of iodinated-LN (T) in a range from 0.1 to 6 nM, both in the presence and absence of a 100-fold unlabeled LN excess; thus it was possible to determine the amount of labeled LN specifically bound to M2R cell surface (B). On plotting B versus T, a binding isotherm was obtained. This isotherm was consistently represented by an S-shaped curve (Fig. 1 C), suggesting multiple interactions between M2R and LN, as discussed above.

Reliable measurement of some parameters as Kd (the dissociation constant at equilibrium), depends on linearization algorithms. These procedures allow a good fit and therefore an adequate precision on parameters determination. Scatchard analysis is the simplest and most widely used model for binding isotherm linearization (Scatchard, 1949, *Ann. N. Y. Acad. Sci. 51*: 61). On plotting the binding isotherm data on Scatchard coordinates, a bi-modal curve was obtained, suggesting the existence of at least two distinct binding sites to LN on M2R cells, one of which presenting positive cooperativity, as discussed elsewhere (M. V. Marquezini et al., 1987, *Cell Diff., 20*: 23S). However, the model which Scatchard analysis is based upon, does not account for the possibility of cooperativity.

Another algorithm, known as Hill analysis, foresees the possibility of cooperativity (Wilhelm & Norman, 1985, *J. Biol. Chem., 260*: 10067). In this model, linearization is obtained by plotting log [B/(Bm-B)] versus log F, where Bm is the maximal binding capacity and F is the amount of free ligand at equilibrium (F = T − B). Bm can be estimated by a semilogarithmic analysis, by plotting B versus log F. This curve is generally represented by a sigmoidal curve, at inflexion half receptors must be occupied (Klotz, 1982, *Science, 217*: 1247). The latter analysis allows us to determine a composite Kd. Indeed, operationally, the concept of Kd can be stated as the amount of ligand necessary to occupy 50% of total amount of receptors, however it is not possible to discriminate distinct receptors using this approach.

Analyzing binding data on Hill coordinates (Fig. 1C, insert), it was possible to identify 2 components. The first one is represented by a single site (as its Hill coeficient, nH = 1) presenting a Kd of

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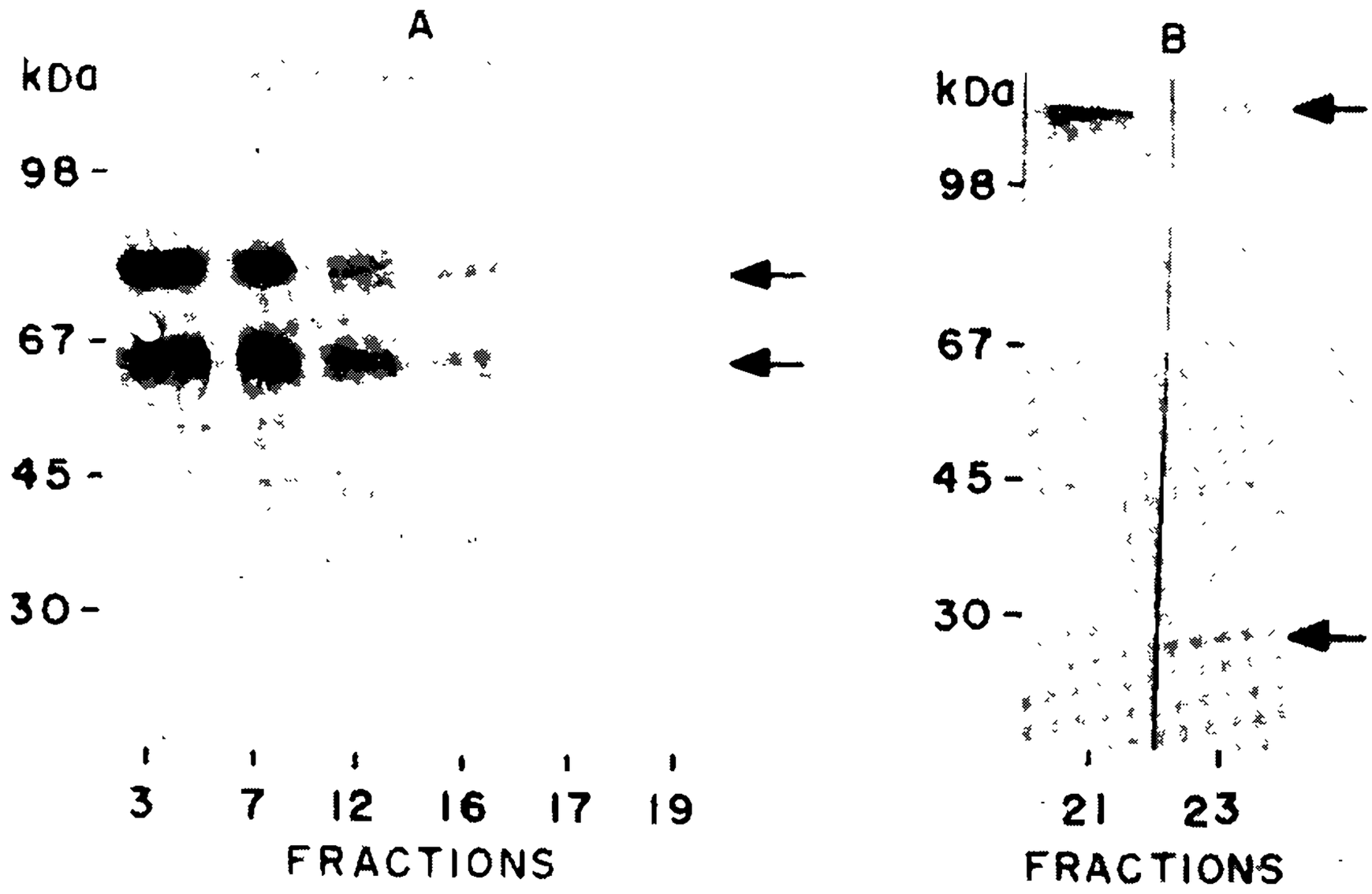

Fig. 1: laminin receptors heterogeneity in M2R cells. A and B: elution profile from a LN-Sepharose column. C: LN binding isotherm (▲, total bound; O, nonspecific bound) and Hill analysis (insert).

3.55 nM; and the second one, represented by about 3 sites (nH = 2.75), presenting a composite Kd of 8.08 nM. This second component presents positive cooperativity (nH 1), that could be explained by either conformational alterations of a single receptor or clustering of distinct LN receptors.

M2R cell membranes can be considered as heterogeneous matrices of laminin receptors. Indeed, accumulating evidences point to a great diversity among laminin binding glycoproteins or glycoconjugates, in the same cell type. In this regard, radioligand binding approach would be adequate to determine binding parameters, only when specific features in the nature of the interaction could be experimentally assessed. At last, as discussed above, a good approximation on global binding parameters could be achieved by using the algorithm suggested by Klotz.