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Different cellular receptors for human placental laminin and murine EHS laminin

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Human placental laminin purified without the use of proteases promotes similar levels of cell attachment to murine EHS laminin. The major HT1080 cell binding site of human placental laminin is thermally less labile than that of EHS laminin. Monoclonal antibody GOH3, which recognises the integrin α6 subunit, inhibits HT1080 and B16 cell attachment to EHS laminin but not to human placental laminin, indicating that the cells use different receptors to bind to these two types of laminin. Antibodies P1E6 and P1B5, which recognise the integrin α2 and α3 subunits respectively, do not affect HT1080 cell binding to either type of laminin.

Basement membrane; Integrin; Cell attachment; Laminin

1. INTRODUCTION

The basement membrane glycoprotein laminin affects cell attachment, polarisation, migration and differentiation [1]. The most commonly studied isoform, murine EHS laminin, has an A (440 kDa), a B1 (220 kDa) and a B2 (220 kDa) subunit [2], although laminin isoforms with alternative subunits have also been described [3–5]. Different cell types can use a variety of receptors, including integrins, to interact with different sites on the EHS laminin molecule [1,6–9], although, since multiple laminin isoforms exist, the physiological relevance of such interactions is not clear. To date the only cell-binding studies with laminin from another source have utilised human placental laminin prepared using pepsin digestion [10–12], but with EHS laminin such treatment reveals a cryptic binding site not present in the intact molecule [13] and destroys the long-arm binding site [14]. Here we directly compare cell attachment to murine EHS laminin and human placental laminin purified without the use of proteases. Our results suggest that for a given cell type, different receptors mediate binding to laminin molecules of different origins.

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Abbreviations: EHS, Engelbreth-Holm-Swarm; ELISA, enzyme-linked immunosorbent assay

2. MATERIALS AND METHODS

Human placental laminin was purified as previously described [15], but with an extra purification step on gelatin-Sepharose to remove fibronectin. Murine EHS laminin was purified as described [16]. A monoclonal antibody against the cell binding region of human fibronectin (Telios, US) showed no reactivity by ELISA with either laminin preparation (not shown).

Cell attachment assays were carried out as described [14], except numbers of adherent cells were assayed while still on the plates by measuring hexosaminidase activity [17]. Human laminin concentrations were estimated from their absorbance at 280 nm normalised to an EHS laminin standard. Cell attachment is expressed as % of cells added to the wells, with error bars showing the standard deviation ($n = 3$–4). For inhibition experiments with anti-laminin antisera, coated and blocked plates were incubated with the indicated dilutions of antiserum for 1 h at 37°C, after which the plates were washed with phosphate-buffered saline and the cells added. For inhibition experiments with anti-integrin monoclonal antibodies, cells were incubated with either GOH3 (rat anti-mouse α6 [15], gift of A. Sonnenberg, Amsterdam, The Netherlands), P1B5 (mouse anti-human α3 [19], Telios, US) or P1E6 (mouse anti-human α2 [19], Telios, US) for 1 h at 37°C, before being added to the plates. Heat treatment of laminin was carried out as described [14].

3. RESULTS AND DISCUSSION

The abilities of human placental laminin and murine EHS laminin to promote attachment of RuGli (rat glioblastoma), HT1080 (human fibrosarcoma) and B16 (mouse melanoma) cell lines were compared (Fig. 1). All three cell lines attached to both types of laminin and slightly lower amounts of human placental laminin than EHS laminin were required to support the same level of binding. Maximum levels of cell binding for RuGli (70–80%) and for HT1080 (40–50%) cells were similar on both types of laminin, and for B16 cells were slightly higher on the human laminin (60%) than on EHS laminin (45%).

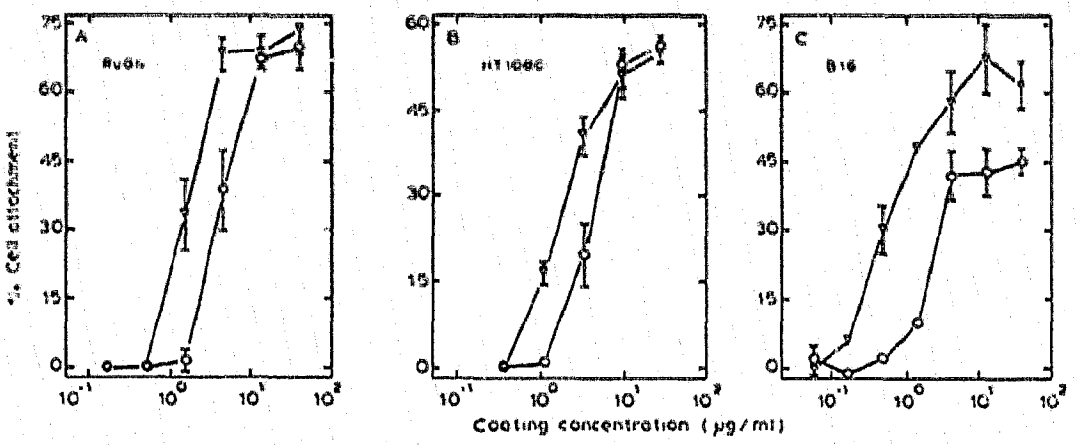

Fig. 1. Attachment of RuGli (A), HT1080 (B) and B16 (C) cells to plates coated with murine EHS laminin (○) and human placental laminin (▽).

Previous studies have shown that the long-arm region (E8) of EHS laminin contains a major cell-attachment site and is involved in many of laminin's biological functions [14,20-23]. This region of EHS laminin is heat-sensitive, with cell attachment activity being lost after heating to 60-70°C [14]. Human placental laminin was more resistant to heat-treatment, with temperatures of 80-90°C being required to abolish HT1080 cell attachment (Fig. 2), indicating either that the major cell binding region of human placental laminin is not its long-arm region or that its long-arm region is thermally more stable and therefore structurally different from that of EHS laminin. Further evidence that the major cell binding regions of the two types of laminin are distinct was provided by the finding that a rabbit antiserum against EHS laminin could block binding of Rugli cells to EHS laminin but not to human placental laminin (Fig. 3) although this antiserum cross-reacted with the human laminin by ELISA at these concentrations (not shown).

Many cell lines have been shown to use an α6-subunit containing integrin to bind to EHS laminin via the E8 region [6-8]. We therefore tested the ability of the monoclonal antibody GOH3 [18], which can block α6-integrin-mediated cell attachment to EHS laminin [6-9], to block B16 and HT1080 cell binding to human placental laminin. GOH3 completely inhibited binding of both cell lines to EHS laminin but had no significant effect on the binding of the same cells to human placental laminin (Fig. 4). Therefore, the cells are using different receptors to bind to these two types of laminin. At present it is unclear whether the differences in the cell binding sites of the two laminin molecules are due

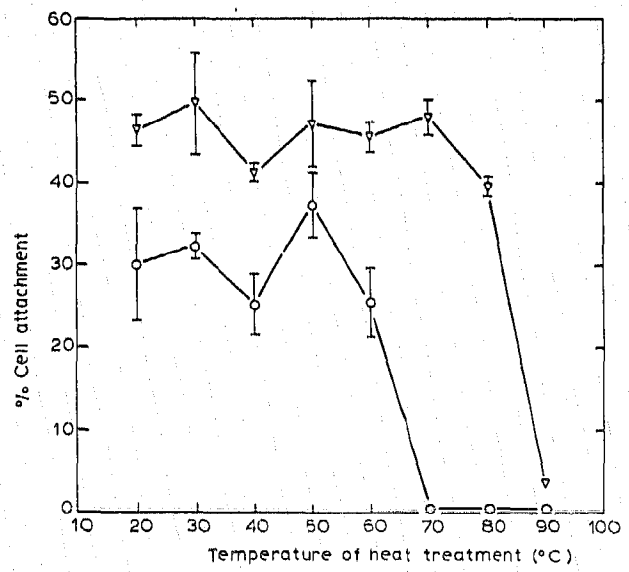

Fig. 2. HT1080 cell binding to heat treated EHS laminin (○; 50 μg/ml) and human placental laminin (▽; 10 μg/ml).

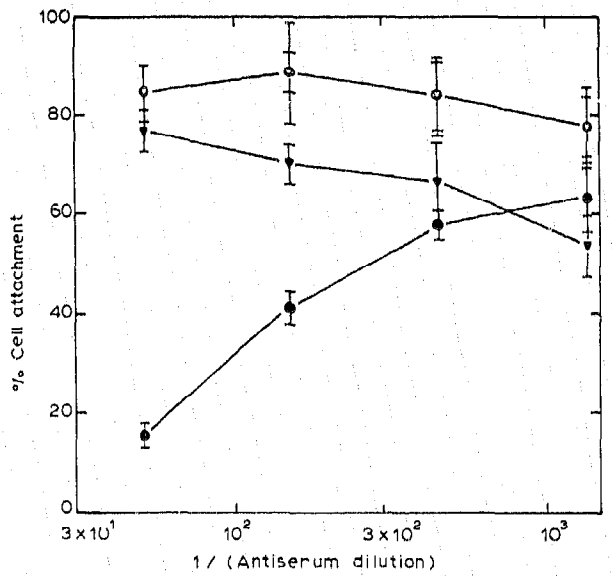

Fig. 3. Wells coated with murine EHS laminin (○●; 5 μg/ml) or human placental laminin (▽▼; 2 μg/ml) were incubated with rabbit anti-EHS laminin antiserum (closed symbols) or normal rabbit serum (open symbols). After removing excess antiserum, RuGli cells were allowed to attach.

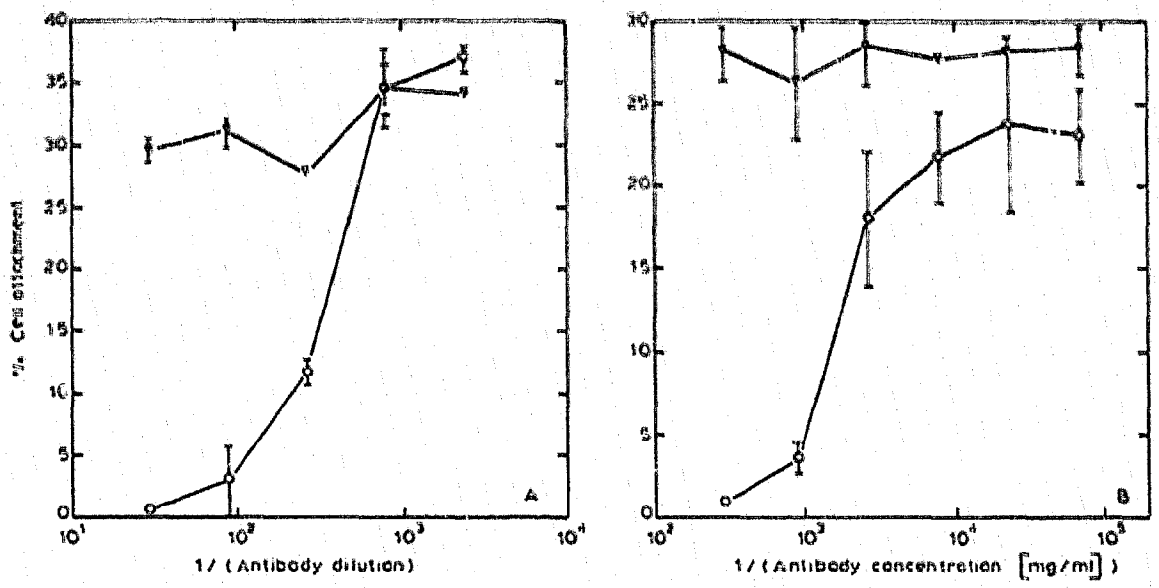

Fig. 4. HT1080 (A) or B16 cells (B) were incubated with GOH3 (anti-α6) tissue culture supernatant (A) or purified antibody (B) then added to wells coated with murine EHS laminin (○; A, 10 μg/ml; B, 3 μg/ml) or human placental laminin (▽; A, 10 μg/ml; B, 3 μg/ml).

to species or tissue heterogeneity, or to peculiarities of tumour-derived laminin. Tissue-specific laminin isoforms have been described [3–5] and the human laminin used in this study contains a subunit which is antigenically unrelated to the EHS laminin subunits (unpublished observations).

Since HT1080 cells express high levels of the integrin subunits α2 and α3 (unpublished results), both of which have been reported to act in combination with β1 as laminin receptors [10–12,24], we also studied the affect of monoclonal antibodies against these subunits on HT1080 cell binding to human placental laminin. P1E6 (anti-α2 [19]) blocked HT1080 cell binding to human collagens I and II (Fig. 5A) but had no effect on their binding to either murine EHS or human placental laminin (Fig. 5B). This suggests that the HT1080 cells are not using an α2-integrin as a major human placental laminin receptor and eliminates the possibility that collagen contamination of the human laminin preparation is responsible for its cell adhesion-promoting activity. Previously observed α2-mediated cell attachment to laminin appears to be cell-type specific [12,24]. We observed no significant inhibition of HT1080 cell attachment with P1B5 (anti-α3) on any of the ligands tested (human and murine laminin, collagens I and II (Fig. 5C,D) and fibronectin (not shown)) in contrast to previous reports [19]. Thus, we were unable to draw any conclusions about the role of α3-integrins, which have been shown to bind to pepsin-digested human placental laminin [10,11], in mediating HT1080 cell attachment to intact human placental laminin. In addition, we cannot rule out the possibility that HT1080 cells are using an α6-integrin as a minor human placental laminin receptor in combination with another receptor. Further experiments to identify the HT1080 receptor for human placental laminin are currently being carried out.

This report indicates for the first time that cells may use a distinct receptor repertoire to bind to laminin molecules of different origins. The interaction of α6 integrins with laminin has been demonstrated for several cell types [6–9] and has been shown to be functionally

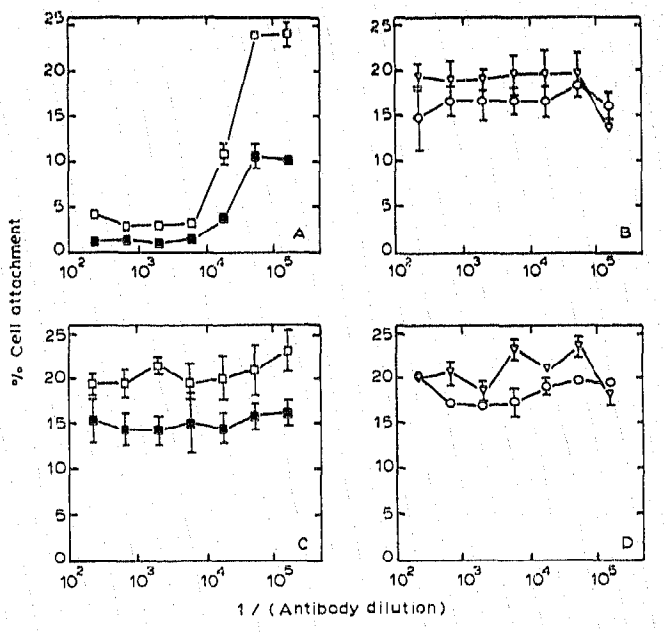

Fig. 5. HT1080 cells were incubated with P1E6 (anti-α2) ascites (A,B) or P1B5 (anti-α3) ascites (C,D) then added to wells coated with 3 μg/ml murine EHS laminin (○), human placental laminin (▽), human collagen I (■) or human collagen II (□).

significant in the developing mouse kidney [25]. Our results demonstrate that this interaction may not be generally applicable to all laminin isoforms and indicate that experiments using laminin of appropriate origin will be required to provide accurate in vitro models of cell attachment and behaviour which occur in vivo.

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