Monoclonal antibodies which prevent experimental lung metastases

Interference with the adhesion of tumour cells to laminin

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Cellular adhesion is important during metastasis, as metastatic cells must escape from the primary site into lymph and blood systems, there to adhere specifically to sites in distant organs. We have recently selected monoclonal antibodies which prevent adherence of B16 mouse melanoma cells to tissue culture dishes, and also markedly reduce experimental lung metastasis in mice when injected before or with the tumour cells. Here, we investigated which step in the metastatic process may be affected by the antibodies. The possible inhibitory effect of antibody on tumour cell adherence to vascular endothelial monolayers and to purified components of the underlying extracellular matrix — fibronectin, laminin and collagen type IV — was studied using in vitro assays. We found that the antibodies significantly blocked attachment to laminin, suggesting that specific basement membrane components play an important role in attracting or otherwise modifying the behaviour of metastatic tumour cells.

1. INTRODUCTION

Cell surfaces are crucial in all cell adhesion processes, and investigations with a variety of tumour models have indicated that cell surfaces play an important role in metastasis as well. For instance, the high metastatic potential of mouse B16 melanoma subline F10 could be transferred to low metastatic variants (F1) through fusion with membrane vesicles from F10 [1]. Similarly, several surface parameters of metastatic tumour cells such as sialylation of surface components, lectin binding, and presence of surface proteases, have been explored, and a correlation with the metastatic potential has often been observed (see [2–4]).

A promising approach to the identification of surface components involved in cell adhesion is the production of antibodies, either polyclonal or monoclonal, which inhibit cell–cell or cell–substrate interactions [5–8]. We have recently generated a series of monoclonal antibodies against B16 mouse melanoma cells by immunizing syngeneic C57BL/6 mice and fusion of the splenocytes with NS-1 myeloma cells. The selected antibodies inhibit the in vitro adhesion of B16 melanoma cells and react with various other murine and human tumour and embryonal cell lines but not with untransformed cells [8,9]. In vivo, three of the antibodies (16/43, 16/77, 16/82) prevented lung colonization of highly metastatic B16 melanoma cells [8]. To examine with which step of the metastatic cascade these antibodies might interfere in vivo, we studied their effect on various cellular and acellular surfaces in vitro.
2. EXPERIMENTAL

2.1. Isolation of B16 subclones

B16 subclones of different metastatic potential were produced by limiting dilution and were tested by intravenous injection (2 \times 10^5 cells per C57BL/6 mouse) as in [8]. For description of the human tumour cell lines see [9].

2.2. Adhesion assay

Bacterial plastic dishes were coated at room temperature for 2h with laminin (15 \mu g/ml), fibronectin (5 \mu g/ml), or collagen IV (5 \mu g/ml) in phosphate-buffered saline. Human fibronectin was prepared as in [10], laminin and collagen IV were generous gifts from Dr R. Timpl (Munich). Endothelial cells from bovine aorta were seeded at 1000 cells/well on Terasaki plates and were allowed to reach confluency [11]. Trypsinized B16 mouse melanoma and human tumour cells (400 cells/50 \mu l for coated plastic dishes and 200 cells/20 \mu l for endothelial monolayers) were plated in hybridoma supernatants (vs control medium) for 30 min at 37°C and attached cells were counted as in [8,11].

3. RESULTS

B16 melanoma cells in the presence or absence of antibodies were seeded: (i) on bacterial plastic dishes coated with components of the basal lamina such as laminin, fibronectin or collagen IV; or (ii)

Fig. 2. Effect of monoclonal antibodies on the adhesion of B16 melanoma cells to cellular and acellular surfaces. Trypsinized B16 melanoma cells of different metastatic potential were plated in hybridoma supernatants (vs NS-1 control medium) on different substrates, and attached cells at various times were counted as in [8,11]. Inhibition of adhesion produced by the 3 antibodies (average data from 2 different experiments) are expressed in bars (% inhibition in comparison to NS-1 control medium) as indicated by the hatched area (antibody 16/43), black area (antibody 16/77), and dotted area (antibody 16/82). The different B16 melanoma lines are: (a) clone 129 (340 lung metastases in C57BL/6 mice); (b) clone 139 (164 lung metastases); (c) clone 164 (40 lung metastases); (d) F10 (50 lung metastases); (e) F1 (5 lung metastases); (f) clone 56 (no metastases). Metastasis formation was analysed by intravenous injection of 2 \times 10^5 cells into mice as in [8].
on confluent monolayers of aortic endothelial cells grown in tissue culture. B16 melanoma cells attached to all substrates within 30 min (fig. 1).

Examination of the 3 antibodies revealed that they were highly active in preventing the adhesion of melanoma cells to laminin (fig. 2). In general, inhibition of 30–90% was observed on laminin, whereas the corresponding values on fibronectin and collagen IV were between 0–25%. The antibodies were virtually inactive when B16 cells adhered to aortic endothelial monolayers (fig. 2) or to extracellular matrix of corneal endothelial cells (not shown). We have also compared the effectiveness of the antibodies on B16 sublines of different metastatic potential. In all cases the inhibition was most effective on laminin (fig. 2), thus no clear correlation was found between metastatic potential of the cells and the activity of the antibodies. High activity of the antibodies on laminin substrate was also found with different human tumour cell lines (fig. 3).

4. DISCUSSION

The finding that monoclonal antibodies can reduce experimental lung lesions in mice and also reduce the adhesion of tumour cells to laminin has implications for our understanding of the process of metastasis and the mechanism of interference by antibodies. The formation of blood-borne metastases falls into several distinct steps: circulating tumour cells must adhere to the endothelial surfaces of the target organs, they then seem to induce endothelial cells to retract, and they must finally break through the underlying basement membrane (which contains laminin) and enter mesenchymal tissue [3,4]. Thus, metastatic tumour cells are able to adhere both to other cells (cell–cell interaction) and to acellular components of the basement membrane (cell–substrate interaction). It is clear from previous studies that different cellular mechanisms and also different cell surface molecules are responsible for these two types of interactions [5–9,12]. As judged from the presented data, our monoclonal antibodies seem to interfere with a kind of cell–substrate interaction; the antibodies were initially selected for such an activity (on tissue culture plates, [8]), and they have now been shown to be highly active on laminin substrates. Anti-B16 antibodies which did not inhibit or only moderately affected metastasis formation in C57BL/6 mice [8] showed a more complex inhibition pattern in vitro. For instance, antibody 19/1 (in vivo negative) inhibits attachment on laminin as well as on collagen IV, whereas antibodies 16/51 and 16/56 (in vivo slightly positive) also specifically blocked adhesion to laminin, but to a lower degree (only 15–40% with clones 129 and 139). This suggests that the interaction between metastatic tumour cells and a laminin-containing substrate, e.g., in the vascular endothelial basement membrane, might be important at one point of metastasis.

Several laboratories have recently shown that various tumour and non-tumour cells express laminin receptors on their surfaces [13–15]; these receptors have $M_r$ values of 60–70000 on SDS-polyacrylamide gel electrophoresis. However, our antibodies react with components of $M_r 40–50000$ [9]. Thus, it is likely that they interfere with adhesion through a surface component different from the commonly recognized laminin receptor. Alter-

Fig. 3. Effect of monoclonal antibodies on the adhesion of human tumour cells to cellular and acellular surfaces. Experiments were performed as described in the legend to fig. 2. The cells were: (a) TR126, a freshly isolated tongue carcinoma cell line; (b) TuWi, from Wilm tumour; (c) Tagli, a freshly isolated glioblastoma cell line (see [9]).
natively, the antibodies might prevent adhesion of metastatic tumour cells to laminin by indirect signaling mechanisms (for a discussion of these possibilities see [7]). Interestingly, no difference between high and low metastatic B16 variants in their adhesion to laminin and in the antibody effects in vitro could be seen (fig.2, for a discussion of the properties of such variants see [2,3]). This indicates that the binding of the tumour cells to laminin-containing structures in vivo is surely but one step in metastasis formation. However, as others have suggested as well [16], it seems to be one with a great potential for anti-metastatic therapy.

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