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# αvβ5-Integrins mediate early steps of metastasis formation

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**Abstract**

Tumour cell adhesion within the microvasculature of host organs, its stabilisation and cell invasion into the host organs, appear to be important steps in the formation of distant metastases. Intravital fluorescence-video microscopy was used to investigate the early steps in metastasis formation of colon carcinoma cells within the liver, which is the main target organ of colorectal carcinomas. The involvement of αv-integrins was analysed *in vivo* using HT-29 cells after treatment with different function-blocking antibodies [pan-αv ($n = 9$ animals), specific αvβ3 ($n = 8$ animals) and αvβ5 ($n = 8$ animals)] or linear Arg–Gly–Asp (RGD)-containing peptides (RGD-peptides) ($n = 6$ animals). Treatment with anti-αv and anti-αvβ5 antibodies resulted in significantly ($P < 0.001$) decreased tumour cell adhesion *in vivo* within the hepatic microvasculature. Cells treated with anti-αvβ3 antibodies or unspecific immunoglobulin-G (IgG) did not show significant changes in their adhesive properties. Furthermore, inhibition of cell adhesion was achieved by linear RGD-peptides in a dose-dependent manner. Relative numbers of migrated cells were not affected by any of the treatments. These results suggest that αv-integrins, especially αvβ5, can influence the ability of circulating tumour cells to adhere within the hepatic microvessels. In contrast, migration of adherent cells into the liver parenchyma was not affected by αv-integrin inhibition. Our findings support the hypothesis that specific interactions between circulating tumour cells and host organs are required for organ-specific tumour cell arrest.


*Keywords:* αv-Integrin; Liver metastasis; Intravital microscopy; Colon carcinoma; Cell adhesion; Invasion

## 1. Introduction

The spread from primary tumours to distant organs is the life-limiting aspect of most malignant diseases. This process consists of a number of sequential, interrelated steps, all of which can be rate-limiting [1]. An important and early step during formation of distant metastasis is the arrest of circulating tumour cells within the host organ [2]. Various types of cell adhesion molecules appear to be involved in the complex processes of metastatic tumour cell adhesion to the microvasculature. These cell adhesion molecules can mediate successful cell arrest, which appears to be dependent on the balance between adhesive and anti-adhesive forces, and the rate at which adhesive interactions are broken [3]. Recent reports suggest that stabilisation of tumour cell adhesion to the microvessels of host organs is important for further stages of secondary tumour formation [4]. In addition to the biochemical interactions between adhesion molecules and their ligands, tumour cell adhesion within circulatory systems appears to be influenced by biophysical factors, such as shear stress caused by fluid flow, and cellular and soluble components of the circulating fluid [5–7].

The liver is the most important host organ for metastasis of colon carcinomas and many other malignancies. The sinusoidal endothelial layer is characterised by an incomplete covering of the microvessel structures, which

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leaves extracellular matrix (ECM) components directly accessible to circulating cells [8]. Endothelial cell fenestrae are grouped in sieve plates and measure approximately 175 nm in diameter, occupying 6–8% of the sinusoidal surface connecting the sinusoidal system with the perisinusoidal space of 'Disse' [9]. The liver is furthermore unique, since it lacks a basement membrane underneath the sinusoidal endothelium [10]. This unique nature of the hepatic ECM is predicted by its special configuration and apparent continuity with the extraparenchymal areas of the connective tissue [11]. In contrast, other epithelial organs have subendothelial basement membranes and a substantial ECM between the endothelial and epithelial cells. In contrast, the liver lobule has only an attenuated ECM, consisting mostly of fibronectin (FN), some type I collagen (C I) and minor quantities of type III, IV, V and VI collagens, tenascin and laminin, whereas vitronectin (VN) is usually not present in the space of 'Disse' [12]. Various studies have shown that interactions between these ECM components within the liver and tumour cells appear to be crucial for the formation of hepatic metastases [13–15].

Integrin expression as the ligands for ECM binding can profoundly influence formation of metastasis in various tumour entities [16,17]. However, the mechanisms by which integrins participate in various steps in metastasis formation *in vivo* are only partially understood [18]. These adhesion molecules appear to be involved in cell adhesion, migration and invasion of the tumour cells, but they can also play an important role in neoangiogenesis and tumour cell proliferation [18]. The αv-integrins have been considered one of the most important group of adhesion molecules for these processes. For example, for initial arrest of breast cancer cells within the lung, αvβ3-integrins were found as mediating adhesion receptors *in vitro* [17]. The same integrin was found to regulate extravasation of Chinese hamster ovary (CHO) cells into the liver parenchyma in an *in vivo* model [19]. Most metastatic melanomas and highly metastatic melanoma cells have high levels of αvβ3-integrin expression, whereas parental and low metastatic cells show low expression of this integrin [20,21]. Furthermore, these integrins appear to be involved in tumour-induced neoangiogenesis that is required at primary and secondary tumour sites [22,23]. Other integrins, such as α5β1-, αvβ1-, α6β4-, αvβ5- or αvβ6-heterodimers, have also been related to metastatic phenotypes [18,24]. For example, decreased expression of α5β1-integrins, but increased expression of α2β1-integrins appears to be correlated with tumourigenicity and metastatic potential [24–26].

Consequently, treatment strategies have been developed that can inhibit integrin-mediated tumour characteristics. For example, reduced growth of malignant melanomas at both primary sites and experimental lung metastasis can be achieved using the anti-αv integrin antibody 17E6 [27–29]. Moreover, neoangiogenesis can be inhibited by antibodies against αvβ3-integrin or using RGD-containing peptides for blocking of integrin-binding sites [30–32]. Although a reduction in microvessel densities in these tumours has been observed [23], the complex cellular functions of αv-integrins suggest that this integrin subgroup might be involved in different steps in the metastatic cascade.

It is the case, however, that most experimental data were obtained using *in vitro* systems that probably simplify biological responses and reduce their complexity within the host organs or using *in vivo* endpoint assays with macroscopic tumours as target structures. These endpoint assays are unable to differentiate between different steps in the metastatic cascade, such as cell adhesion, survival or proliferation. Intravital microscopy technologies have been used recently to investigate metastatic tumour cell adhesion within host organ microcirculation, such as liver and lung [33–37]. In these studies contradictory results were reported regarding the type of entrapment (mechanical entrapment [34–37] versus active cell adhesion [37,38]) and the requirement of invasion into host organ parenchyma (invasion [34,35] versus intravascular proliferation [38]). Using different colon carcinoma cells we observed that circulating tumour cells can arrest in rat liver sinusoids and rapidly extravasate into the liver parenchyma, mainly via active tumour cell adhesions mediated by various cell adhesion molecules [38,39].

Using a direct observation of metastatic tumour cell adhesion and invasion of colon carcinoma cells into the host liver parenchyma by intravital microscopy we investigated the role of αv-integrins in these early steps of metastasis formation. We found that inhibition of these integrins, and especially αvβ5-integrins, significantly inhibited tumour cell adhesion to ECM components *in vitro* and to sinusoidal microvessels *in vivo*, but did not interfere with their migration into the liver parenchyma.

## 2. Methods

### 2.1. Materials and cells

Roswell Park Memorial Institute (RPMI)1640 medium and foetal bovine serum (FBS) were purchased from GIBCO-BRL (Karlsruhe, Germany). The monoclonal antibodies (mAb) against integrins (clone 17E6, pan-αv-inhibition), αvβ3-integrins (clone LM609) and αvβ5-integrins (clone P1F6) have been described previously [28,29]. In some experiments a function-blocking antibody against β1-integrins (clone P4C10, Chemicon, Hofheim, Germany) was used [29]. Unspecific mouse IgG (Sigma, Deisenhofen, Germany) served as control treatment. We have previously demonstrated that this treatment does not interfere with adhesive properties

of HT-29 cells *in vitro* [14]. All other chemicals were also purchased from Sigma.

Parental HT-29 colon carcinoma cells expressing αvβ5-integrins but not αvβ3-integrins were cultured in RPMI1640 medium containing 10% FBS without antibiotics in humidified 5% $CO_2$/95% air at 37 °C. Cells were reconstituted in serum-free medium (RPMI1640, bovine serum albumin (BSA) 1%) for 60 min. In some experiments cells were incubated with the appropriate antibodies (1–3 μg/ml, 60 min) or linear RGD-peptides (0.2–0.5 mM, 60 min), respectively, prior to the assays. This pre-treatment has been shown to interfere with the regulation of cell adhesion to ECM components in HT-29 cells *in vitro* without disturbing their viability [40].

### 2.2. Static adhesion assays

Enzyme-linked immunosorbent assay (ELISA) microplates (Dynatech, Chantilly, VA, United States of America (USA)) were coated with vitronectin (VN, Collaborative Biomedical Products, Bedford, MA, USA) at 4 μg/ml; fibronectin (FN, Sigma, St. Louis, MO, USA) at 10 μg/ml; type I collagen (C I, Cellon) at 10 μg/ml. Blocking of non-specific binding sites was performed with 1.5% BSA (Merck) and serial dilutions of selected antagonists were made on the same plate with adhesion medium (RPMI plus glutamax-I plus 1% BSA). Cells, resuspended in adhesion medium ($1.6 \times 10^6$/ml; 50 μl/well) were added to the wells and the plates incubated at 37 °C for 1 h.

Non-adherent cells were removed by flooding the plates with PBS. Adhered cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. After washing and drying cells, colour was developed with 0.1 M HCl and read in a microtitre plate spectrophotometer at a wavelength of 630 nm. The percentage of cell adhesion inhibition was calculated using wells without antagonists as 100% adhesion. Results presented were calculated from the average of duplicate samples and were representative of several separate assays.

### 2.3. Intravital fluorescence video microscopy

Male Sprague-Dawley rats (weight 200–250 g, Charles River) were cared for in accordance with standards of the German Council on Animal Care, under an approved protocol of the local Animal Welfare Committee. Rats were prepared as described previously [38,39]. After a wide median laparotomy was performed the left liver lobe was careful mobilised without disturbing the hepatic circulation. Using a heated operating table, animals were then fixed under an upright microscope and positioned on their left side. This positioning allowed a partial luxation of the mobilised left liver lobe, which was placed on a specific holder to investigate its lower surface.

An upright epifluorescence microscope (Zeiss, Oberkochen, Germany) was used, with its 20-fold objective located over a glass slip covering the liver surface. The microscope was connected to a video enhancer-zoom lens system and a low-light CCD-video camera (Peiper, Düsseldorf, Germany) allowing real-time imaging via a separate monitor. Fluorescence images were recorded using a timer-containing S-VHS videosystem for further analysis [41].

### 2.4. In vivo observation of metastatic tumour cell adhesion

For intravital observation of adhesive interactions between circulating tumour cells and the hepatic microcirculation, single cell suspensions ($1 \times 10^6$ cells) were injected intra-arterially over 60 s. As previously reported [38], this technique did not interfere with cardiocirculatory or pulmonary functions of the animals.

Various parameters were used for further investigation and semi-quantitative analysis of these interactions. Firstly, occurrence of 'cell rolling' was monitored. Additionally, the latency was measured until the first stable tumour cell adhesions were established within the hepatic microcirculation. The localisation of stable tumour cell adhesions within the vascular tree and in relation to the diameter of the involved vessels was evaluated. Furthermore, remaining blood flow within this vessel or its occlusion was investigated. A semi-quantitative analysis of tumour cell adhesions and extravasation was performed over a 30-min observation period and the numbers of adherent cells were counted for each of the 5-min intervals. Using a standardised procedure, 30 microscopic fields were analysed in each observation period and average numbers of adherent cells, migrated cells and total cells observed for each 5-min observation interval were counted. In addition, the latency of tumour cell invasion into liver parenchyma was determined. The relative migration rates were calculated as percentage of cells within the hepatic parenchyma in relation to the total number of observed cells.

### 2.5. Statistical analysis

Statistical analysis was performed using the StatMost32 statistical program (DataMost Corp., Los Angeles, CA, USA). $P$ values were calculated according to two-sided Student's $t$ -tests for independent samples. Significant differences were accepted for $P < 0.05$.

## 3. Results

### 3.1. In vitro inhibition of αv-integrins

HT-29 cells were able to adhere to several ECM components, for example VN, C I and FN. To elucidate

their inhibitory potential for tumour cell adhesion to those ECM components, different integrin-specific antibodies against αv-integrins were evaluated using microtitre plate assays. Fig. 1 shows the percentage of cell adhesion inhibition caused by these antibodies. HT-29 cells attached to VN mainly by αvβ5 since the specific αvβ5 antibody P1F6 completely blocked this adhesion. In addition, the pan-αv blocking antibody 17E6 is also a blocker of HT-29 cell adhesion to VN. According to the negative αvβ3 expression of HT-29 [28,42], the anti-αvβ3 antibody LM609 did not affect cell adhesion. β1-integrins do not play any role in the adhesion to VN, as suggested by the lack of inhibition of P4C10 (anti-β1 integrin antibody) (Fig. 1(a)).

HT-29 cell attachment to FN can be blocked completely by the pan-αv blocking antibody 17E6, but only partially by the anti-αvβ5 and anti-β1 antibodies; suggesting that HT-29 uses mainly αvβ6 integrins to attach to FN *in vitro* (Fig. 1(b)).

Additionally, HT-29 cells use β1-integrins to adhere to C I, since P4C10 antibody was the only inhibitor that could block this cell adhesion. As expected, these data suggest that αv-integrins may not have a role in the HT-29 cell adhesion to native C I (Fig. 1(c))

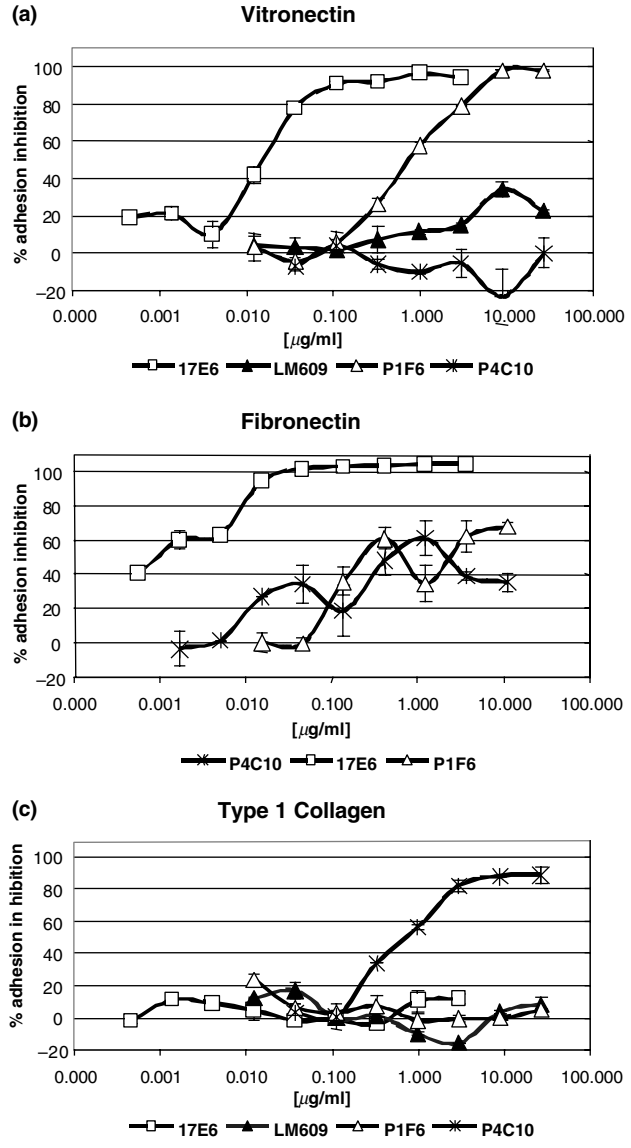


Fig. 1. *In vitro* cell adhesion and inhibition by αv-integrins antagonists. HT-29 cells were allowed to adhere to various extracellular matrix (ECM) components in presence of specific anti-integrin antibodies. Data are expressed as percentage of cell adhesion inhibition to: (a) vitronectin (VN); (b) fibronectin (FN); (c) type I collagen (C I) compared with unspecific immunoglobulin G (IgG)-treated cells. Activities of the inhibitors are: 17E6 (pan-αv), LM609 (αvβ3), P1F6 (αvβ5) and P4C10 (β1).

### 3.2. In vivo metastatic tumour cell adhesion

*In vivo*, circulating HT-29 cells were easily able to pass the liver sinusoids without mechanical entrapment. Within 2 min after injection of the cell suspension, the first cells were observed to arrest within the sinusoids. However, in contrast to adhering leukocytes, rolling of colon carcinoma cells has not been observed. In some cases, adherent cells appeared to lack adhesion stabilisation and lost their adhesive bonds after several seconds, resulting in recirculation of these cells. Adherent tumour cells were located within sinusoidal capillaries with larger diameters than the diameters of the arrested tumour cells. This resulted in a remaining lumen and persistent blood circulation of these capillaries. Small numbers of adherent cells started to migrate rapidly from the intravasal compartment and invaded the liver parenchyma (Fig. 2). The first migrated cells were observed within 5 min after injection of the cell suspension. At the end of the observation period approximately 10% of the observed cells were found within the liver parenchyma. We have previously confirmed early migration into the liver parenchyma using a BrdU-labelling technique and multiple sectioning [42,43].

### 3.3. RGD-peptides inhibit metastatic tumour cell adhesion

For further evidence of specific interactions between circulating tumour cells and hepatic microcirculation that are mediated by integrins we used RGD-peptides, which can inhibit cell adhesion to FN and VN *in vitro* [14]. We have demonstrated previously that LN- and collagen-binding integrins specifically interfere with different steps of host organ colonisation *in vivo* [42].

RGD-peptides showed an unspecific effect on cell adhesion, which was determined by Arg–Gly–Glu–Ser (RGES)-control peptides. However, an additional and concentration-dependent inhibition of cell adhesion, but not relative cell migration, was observed comparable to the effects of specific heterodimer inhibition. Low concentrations of linear RGES and RGD (0.2 μM) had similar effects on *in vivo* cell adhesion. High concentrations of RGD-peptides (0.5 μM) resulted in a further

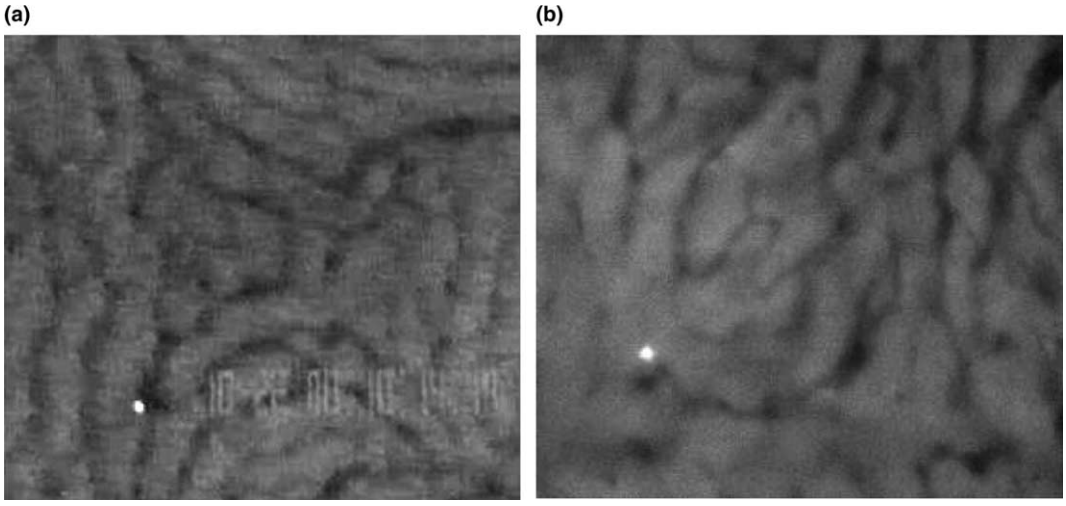


Fig. 2. *In vivo* cell adhesion and invasion into liver parenchyma. After successful arrest within hepatic microvessels fluorescence-labelled tumour cells rapidly extravasated and invaded liver parenchyma. (a) Representative samples are shown where an untreated HT-29 cell became adherent within the sinusoid. (b) Approximately 10% of the cells were able to migrate into the liver parenchyma within the 30 min observation period. Autofluorescence of liver parenchyma enabled differentiation between sinusoids and parenchyma.

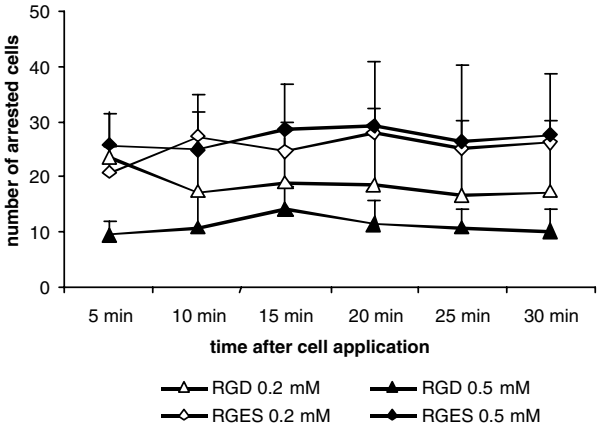


Fig. 3. Arg–Gly–Asp (RGD)-peptides significantly reduce adhesive interactions *in vivo*. Cells were pre-treated with different concentrations (0.2–0.5 μM) of inhibitory RGD-peptides or inactive Arg–Gly–Glu–Ser (RGES)-peptides ($n = 6$ animals in each group). Average numbers of arrested cells (adherent + migrated cells) were counted in each 5 min observation period in 30 standardised microscopic fields for 30 min after injection. A significant and concentration-dependent reduction in adhesive interactions between circulating tumour cells and hepatic sinusoids was observed if adhesion blocking RGD-concentrations were used compared with control peptides.

significant reduction in cell adhesion compared with low concentrations ($P < 0.01$), whereas high concentrations of RGES-peptides did not show concentration-dependent differences (Fig. 3).

### 3.4. Inhibition of different αv-integrins in vivo

The specific inhibition of metastatic tumour cell adhesion within the hepatic sinusoids suggested the involvement of different αv-integrins in this early step of metastasis formation. Therefore, HT-29 cells were treated with a pan-αv-antibody to block all αv-integrins independently from the β-subunit. This treatment resulted in a significant reduction in metastatic cell adhesion (untreated versus pan-αv: $P < 0.001$; IgG versus pan-αv: $P < 0.01$). Although the absolute numbers of migrated cells were also significantly reduced ($P < 0.05$) the relative migration rates were not influenced by blockade of αv-integrins (Table 1, Fig. 4). Lack of 'rolling', passage of sinusoids, localisation of cell arrest and occurrence of recirculation of arrested cells were not influenced by this treatment. Incubation of cells with

Table 1
Relative migration rates of adherent cells[a]

| Interval (min) | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|
| Untreated (%) | 10 ± 10 | 12 ± 7 | 12 ± 5 | 10 ± 3 | 10 ± 4 | 12 ± 5 |
| IgG (%) | 5 ± 6 | 10 ± 9 | 14 ± 7 | 12 ± 3 | 11 ± 6 | 14 ± 6 |
| Pan-αv-integrin (%) | 7 ± 6 | 13 ± 12 | 13 ± 11 | 16 ± 9 | 14 ± 7 | 14 ± 8 |
| αvβ3-Integrin (%) | 3 ± 3 | 8 ± 4 | 12 ± 2 | 12 ± 3 | 13 ± 4 | 11 ± 2 |
| αvβ5-Integrin (%) | 3 ± 4 | 9 ± 5 | 11 ± 3 | 12 ± 3 | 11 ± 4 | 13 ± 4 |

Pre-treatment with different antibodies did not influence relative migration rates.
IgG, immunoglobulin G.
[a] Percentages of migrated cells in relation to the total number of cells with adhesive interactions (relative migration = migrated cells/[adherent cells + migrated cells]∗100%).

unspecific IgG resulted in a slight, but not significant, reduction in cell adhesion compared with untreated cells (data not shown).

As described above, ECM components in the space of 'Disse' can be recognised by αv-integrins. It was hypothesised that these interactions might be important determinants for liver-specific metastasis formation. If αvβ5-integrins were blocked, metastatic cell adhesion was reduced to approximately 50%, similar to the level when using pan-αv-integrins ($P < 0.001$). Similarly, absolute numbers of migrated cells, but not relative cell migration rates, were significantly inhibited. Since HT-29 cells do not express β3-integrins, pre-treatment with anti-αvβ3 antibodies served as a control group and pre-treatment with these antibodies did not significantly affect the early steps of metastatic tumour cell arrest or migration into the liver parenchyma compared with untreated cells or unspecific IgG treatment (Table 1, Fig. 4).

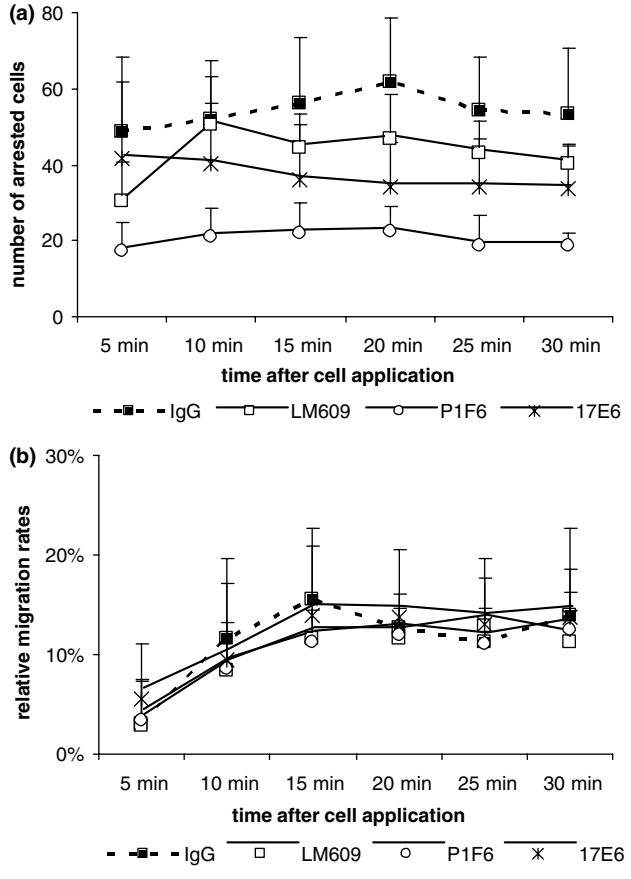


Fig. 4. Effects of αv-integrin inhibition on metastatic tumour cell adhesion *in vivo*. After pre-treatment with anti-pan-αv-integrin (clone 17E6, $n = 8$ animals), anti-αvβ3-integrin (clone LM609, $n = 8$ animals), anti-αvβ5-integrin (clone P1F6, $n = 8$ animals) or unspecific IgG ($n = 9$ animals) cells were injected i.a.; (a) average numbers of arrested cells (adherent + migrated cells) were counted in each 5 min observation period in 30 standardised microscopic fields for 30 min after injection; (b) relative migration rates were calculated (i.e., number of migrated cells/number of arrested cells) in each observation period.

## 4. Discussion

The metastatic process consists of a number of steps that have to be completed successfully for formation of secondary tumours [44]. After tumour cells have entered the circulation directly or indirectly via the lymphatic system they have to arrest in a secondary host organ. It is probable that arrested tumour cells require extravasation for further growth. It has long been accepted that most malignant tumours show an organ-specific pattern of metastasis. For example, colon carcinomas usually metastasise to the liver and lung, but rarely to bone and brain. By contrast, breast carcinomas frequently form metastases in all of these organs. However, experimental data using different types of assays have revealed contradictory results regarding the specificity of initial interactions between host organs and circulating tumour cells. Mechanical entrapment [34,35] and receptor-specific 'seed-and-soil' adhesions [37,38] are currently discussed as determining factors for organ-specific cell arrest.

This study confirmed our previous results, that circulating colon carcinoma cells can arrest within liver sinusoids mediated by specific integrin-dependent tumour cell adhesions and thereby leaving perfused vessel lumen [38,39]. In our experimental system some modifications were used compared with the method used by Chambers and colleagues [34,35] who have reported mechanical cell arrest of circulating tumour cells as the main mechanism. In contrast to this group we used an upright microscope to investigate the liver surface, avoiding additional tissue pressure on the lower side of the liver lobe caused by the weight of the tissues above. This additional tissue pressure might be responsible for increased intravascular pressure and/or reduced size of the microvessels subsequently interfering with tumour cell adhesion. Using different cell lines, the characteristics of adhesion within the liver were found to be independent from the route of cell application. Similar patterns were found if cells were injected intra-arterially, intravenously or intraportally [38]. In this study, we have also demonstrated that rat colon carcinoma cells and human cells of the same tissue type and with comparable tumour characteristics show similar adhesive behaviour within the rat liver [38]. By contrast, using mice systems, mechanical cell arrest was found [45]. Therefore, the choice of experimental system might also count for differences of adhesive interactions observed. In summary, our results and other reports [37] do not support the statement that most circulating tumour cells arrest by size restriction [44], but specific adhesion molecule mediated tumour cell interactions with the metastatic host organs mainly determine organ-specific colonisation.

We have demonstrated recently that circulating colon carcinoma cells adhere selectively in the microcirculation of potential metastatic target organs, such as liver and lung. In contrast, these cells were unable to form stable

cell adhesions within kidneys, which are usually not involved in colon carcinoma spread. In addition, cells with different metastatic potential showed similar cell adhesion within the liver sinusoids, but significantly more highly metastatic cells migrated into the hepatic parenchyma compared with low metastatic colon carcinoma cells (Schlüter and colleagues, data not shown). Furthermore, these adhesive interactions between colon carcinoma cells and the liver sinusoids appear to be specifically mediated by various selectin ligands and some integrin heterodimers. For example, enzymatic removal or functional inhibition of sialyl-Lewis[a] partially reduced tumour cell adhesion. LN-binding integrins (α6β1, α6β4) can also directly mediate cell adhesion, but not migration into the parenchyma. In contrast, collagen-binding integrins (α2β1) mediate migration into the host organ, but not cell adhesion of circulating carcinoma cells [42]. Other cell adhesion molecules, such as α1-, α3-integrins or vascular cell adhesion molecule (VCAM), seem to be not involved in these processes [42].

In this study, we were able to demonstrate an important role of αvβ5-integrins, which also appear to mediate these specific interactions. Inhibition of this integrin heterodimer reduced metastatic tumour cell adhesion, but not early cell migration into the liver parenchyma. Since VN as a ligand for αv-integrins is usually not available in the space of 'Disse', the almost complete loss of adhesive interactions after blockade of integrins with RGD-peptides or anti-αv-integrins suggests that integrin-mediated binding to FN is involved in these interactions. FN is available in the space of 'Disse' in high amounts and relatively accessible to circulating cells due to the endothelial cell (EC) gaps within the liver sinusoids. Furthermore, FN is an ECM component that can provide ligand binding with sufficient rigidity against external forces, such as shear forces by blood flow. Since cell adhesion was affected more by inhibition of all αv-integrins using the pan-αv-antibody (17E6) than by the specific inhibition of αvβ5-integrins (P1F6), we propose that different members of the αv-integrins (β1, β5, β6) appear to be involved in this step of metastasis formation. Some of these data were confirmed using highly metastatic HT-29LMM cells, which demonstrated comparable adhesive behaviour in our model and similar effects of pan-αv-inhibition (data not shown).

It was also reported previously that the pan-αv-antibody (17E6) was more effective in inhibiting melanoma cell adhesion *in vitro* mediated by αvβ5,6-integrins than by αvβ3-integrins. However, specific inhibition of αvβ5-integrins (P1F6) did not affect tumour growth of various adenocarcinomas *in vivo* [29]. These findings suggested an additional role of αv-integrins that form heterodimers with β1-, β5- and β6-subunits *in vivo*. It was hypothesised that αvβ5- and αvβ6-integrins take part in tumour dissemination and metastatic spread rather than in primary tumour growth. Our current results clearly support this hypothesis and show for the first time, that αvβ5- and probably αvβ6- and/or αvβ1-integrins appear to regulate organ-specific metastatic tumour cell adhesion of colon carcinoma cells to FN within the liver microcirculation.

Since HT-29 cells do not express αvβ3-integrins, our study cannot rule out a potential role of this heterodimer in the early steps of metastasis formation. It has been reported that its inhibition can suppress colon cancer metastasis [48], lung experimental metastasis [28] and tumour-induced angiogenesis in mice [46]. This integrin has also been proposed to function in the promotion of extravasation of αvβ3-integrin expressing tumour cells [33]. Furthermore, regulation of metalloproteinases might also contribute to the invasion into the liver parenchyma [47]. In addition, its involvement in growth factor-mediated cell signalling has been discussed [48].

In summary, the ability of adhesion inhibition by anti-integrin antibodies or RGD-peptides supports the 'seed-and-soil' hypothesis in our model. Mechanical arrest would require the complete obstruction of sinusoids by arrested tumour cells, resulting in a loss of perfusion. In addition, mechanical arrest would be affected by adhesion molecules only if there is an interference with biomechanical characteristics of the circulating cell, such as deformability and rigidity [39]. Specific interactions of circulating colon carcinoma cells with FN in the hepatic space of 'Disse' mediated by αvβ5- and possibly αvβ1,6-integrins appear to be important determinants for metastatic tumour cell arrest with the liver sinusoids.

## Conflict of interest statement

None declared.

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