# Ultrastructural localization of integrin subunits α3 and α6 in capillarized sinusoids of the human cirrhotic liver

**F. Quondamatteo[1], C. Kempkensteffen[1], N. Miosge[1], A. Sonnenberg[2] and R. Herken[1]**
[1]Department of Histology, University of Goettingen, Goettingen, Germany and
[2]Division of Cell Biology, The Netherland Cancer Institute, Amsterdam, The Netherlands

**Summary.** Normal liver sinusoids are not lined by a basement membrane (BM). In contrast, in the course of development of liver cirrhosis, a structured BM is formed de novo in the space of Disse. This BM contributes to the inhibition of the metabolic function of the liver but the pathogenic background of the formation of this perisinusoidal BM is still unclear. Integrins of the ß1-class are generally essential for BM stability and some of them (such as α2ß1, α3ß1 and α6ß1) appear de novo in the perisinusoidal space of the cirrhotic liver. Their cellular distribution in capillarized sinusoids as well as the correlation between their cellular distribution and the formation of the microvascular BM in the cirrhotic liver has not been shown at the ultrastructural level. In the present work we aimed to clarify this issue. We focused on integrins α3ß1 and α6ß1 and localised them ultrastructurally in human cirrhotic liver microvessels using postembedding immunogold which allows the ultrastructural localization of antigens with high resolution in the tissue. The newly formed basement membrane of capillarized sinusoids was visualized by means of fixation with addition of tannic acid, which enables the visualization of structures of the extracellular matrix with the highest resolution. Also, we carried out laminin detection using postembedding immunogold. Our results show that both α3ß1 and α6ß1 are expressed on the surface of both hepatocytes and endothelial cells, i.e. on both sides of the newly formed basement membrane. This latter shows zones of higher density both in close proximity to the endothelial and to the hepatocytic surfaces which resemble laminae densae. We propose that hepatocytes and endothelial cells may, therefore, by expressing such integrins, contribute to the formation of this pathological BM in the microvessels of the human cirrhotic liver. On stellate cells, which are major producers of BM components, both integrins α3ß1 and α6ß1 were also localized.

*Offprint requests to:* Fabio Quondamatteo, Dept. of Histology, University of Goettingen, Kreuzbergring 36, D-37075 Goettingen, Germany. Fax: +49-551-397067. e-mail: fquonda@gwdg.de

## Introduction

One of the unique features of the hepatic perisinusoidal matrix is that although it contains basement membrane components, such as type IV collagen and perlecan (Hahn et al., 1980; Murdoch et al., 1994), there is no structured basement membrane (Schaffner and Popper, 1963). However, in the course of liver cirrhosis, a structured basement membrane develops in sinusoids (Schaffner and Popper, 1963). This pathological basement membrane contributes to the inhibition of the metabolic function of the liver (Martinez-Hernandez and Martinez, 1991). The pathogenic background of basement membrane formation in the hepatic perisinusoidal space is still unclear. It is known that basement membrane components in a free solution in vitro can self-assemble and thus polymerize to form a basement membrane (Yurchenco, 1994). On the other hand, it has been well demonstrated that in vivo (Kreidberg et al., 1996; Di Persio et al., 1997; Brakebusch et al., 2000; Raghavan et al., 2000) ß1-class integrins are necessary for basement membranes to be structured and stabilised. In liver cirrhosis in humans, the formation of a basement membrane is not the only change in the microvessels. Alterations in the composition of the matrix of sinusoids and modifications in the expression of integrins in the adjacent cells also occur (Scoazec, 1995). Some integrin subunits, e.g. ß1, together with alpha subunits such as α1, and, therefore, integrin α1ß1, are present in both normal sinusoids and in cirrhosis (Couvelard et al., 1993). In contrast, other subunits, e.g. α2, α3 and α6, and, therefore, the laminin receptors α2ß1, α3ß1 and α6ß1, are absent from normal sinusoids in the liver but are de novo expressed in cirrhotic ones (Couvelard et al., 1993). It is, in fact, conceivable that these changes in integrin expression may contribute to the formation of

the cirrhotic basement membrane in capillarized sinusoids. In this context, the cellular localization of such integrins may help to clarify which of the cells adjacent to the newly formed basement membrane are involved in its formation and stability. The study of the ultrastructural localization of these integrin subunits expressed de novo in capillarized sinusoids is particularly interesting from the point of view of basement membrane formation in contrast, for example, to other subunits, such as ß1 or α1, which are expressed both in normal and in diseased liver (Couvelard et al., 1993). This knowledge would contribute considerably to our understanding of the molecular mechanisms which regulate de novo formation of the perisinusoidal basement membrane in liver cirrhosis in humans. In the present study, we focused on the integrins α3ß1 and α6ß1 and aimed to correlate their ultrastructural distribution in human liver capillarized sinusoids with the newly formed basement membrane in capillarized sinusoids. This latter was visualized by means of fixation with addition of tannic acid which enables visualization of basement membranes (and extracellular matrix in general) with high resolution in ultrathin sections (Herken and Barrach, 1985; Quondamatteo et al., 1998). Also, laminin detection was carried out as a marker of sinusoidal capillarization (Hahn et al., 1980). Integrin as well as laminin distribution was studied by means of postembedding-gold immunohistochemistry which enables the detection of antigens in ultrathin sections with a higher resolution and higher sensitivity in comparison to pre-embedding techniques (Herken et al., 1987; Herken and Miosge, 1991).

**Materials and methods**

*Tissue studied*

Specimens from cirrhotic livers explanted from nine patients undergoing liver transplantation in the Department of Transplantation Surgery of the University of Goettingen were collected according to the Guidelines of the Ethics Committee of the Faculty of Medicine of the University in Goettingen. The cirrhosis was either due to alcoholism (n=2), was secondary to B-hepatitis virus infection (n=1), secondary to C-hepatitis virus infection (n=2), was a biliary cirrhosis (n=1), or was of unclear ethiology (n=2).

During preparation, fragments for examination were taken from at least three different regions from each specimen for each fixation/embedding protocol. After appropriate fixation (see below) the tissue was embedded in epon for an optimal basement membrane visualization or in LR-Gold resin for the immunohistohemical detection of the integrin subunits and of laminin molecules.

*Embedding in Epon*

Liver specimens were obtained immediately after removal of the liver and cut into pieces (1mm$^3$) in 0.1 M phosphate-buffered saline. The tissue was fixed for 90 min in a solution of 1% tannic acid + 1% glutaraldehyde in 0.1M Sörensen's buffer (pH=7.4). Tissue fragments were washed for 20 min in 0.15 M phosphate-buffered saline and subsequently treated with 1% osmium tetroxide in 0.15 M phosphate-buffered saline for 2 h. Specimens were then dehydrated in a graded ethanol series and embedded in epon. For orientation purposes, semithin sections were cut and stained with toluidine blue. Ultrathin sections were then cut with a Reichert-Jung ultramicrotome, collected on formvar-coated copper grids, stained with uranyl acetate for 10 min and with lead citrate for 7 min and examined with a Zeiss EM 109 or a LEO 906E electron microscope.

*LR-Gold embedding*

Liver specimens were obtained immediately after removal of the liver and cut into pieces (1mm$^3$) in 0.1 M phosphate-buffered saline. The tissue was then fixed in 4% paraformaldehyde + 0.5% glutaraldehyde for 30 min. Thereafter, free aldehydes were washed out by incubating the tissue for 45 min with 10mM $NH_4Cl$ at 4 °C, and the tissue was dehydrated in a graded ethanol series up to 70% ethanol. The tissue was then infiltrated with the hydrophilic resin LR-Gold (London Resin, Reading, UK) for 1h at -25 °C, then with LR-Gold supplemented with 0.8% of the light-sensitive initiator benzil overnight at -25 °C in the dark. Polymerization was carried out at -25 °C for 24 h under the light of a 500W halogen lamp.

*Anti-laminin antibodies and antibodies against integrin subunits α3 and α6*

Anti-laminin antibodies were purchased from Sigma (Munich, Germany). Anti-laminin antibodies were polyclonal, raised in rabbits using laminin-1 as antigen (α1, ß1, γ1, Burgeson et al., 1994) and affinity purified.

Antibodies against the integrin subunits α6 (GoH3 rat monoclonal antibody) and α3 (J143 mouse monoclonal antibody) were obtained from hybridoma supernatants (Kantor et al., 1987; Sonnenberg et al., 1987). These were concentrated by means of 45% ammonium sulfate (3.9 M) precipitation at 4 °C. The presence of immunoglobulins was ascertained by SDS-page electrophoresis before antibodies were used for the immunohistochemical detection of integrin subunits.

*Postembedding gold immunohistochemistry*

Light microscopy

Semithin sections (1 µm) were cut using a Reichert-Jung ultracut microtome, collected on microscope slides (Super Frost*/Plus, Menzel, Braunschweig, Germany) and dried for 2 h at 40 °C. The sections were rehydrated with double-distilled water and then treated with Lugol-

solution for 5 min. Thereafter, the slides were dipped in 4% sodium thiosulfate for 20 sec and washed for 2 min in double-distilled water. The sections were predigested with ficin (24 mg/ml in double-distilled water) for 15 minutes at room temperature and unspecific binding sites were blocked with 1% BSA-TBS for 10 min at room temperature. Sections were subsequently processed for the immunohistochemical detection of laminin molecules and of integrin subunits α3 and α6:

*Laminins*. Semithin sections were incubated with the primary antibody 1:10 in 0.05 M Tris-buffered saline (pH=7.4) for 90 min at room temperature. After having been washed in Tris-buffered saline, sections were incubated with a goat anti-rabbit antibody (Medac, Hamburg, Germany) 1:200 in 0.05 M Tris-buffered saline (pH=7.4), for 20 min at room temperature. The secondary antibody was coupled to 8 nm colloidal gold particles in our laboratory (De Mey et al., 1981). Colloidal gold particles of 8 nm were obtained by adding 1 ml of 1% tetrachlorauric acid to 106 ml of 2.2 mM sodium citrate.

*Integrin subunit α3*. Semithin sections were incubated with gold-labeled J143-antibody for 2 h at room temperature, diluted 1:100 in 0.05 M Tris-buffered saline (pH=7.4). Primary antibodies were directly coupled to 8 nm colloidal gold particles in our laboratory (De Mey et al., 1981).

*Integrin subunit α6*. Semithin sections were incubated with gold-labeled GoH3-antibody for 2h at room temperature, diluted 1:100 in 0.05 M Tris-buffered saline (pH=7.4). Primary antibodies were directly coupled to 8 nm colloidal gold particles in our laboratory (De Mey et al., 1981).

Colloidal gold was visualized in semithin sections by silver enhancement. Following the antibody reactions, the sections were rinsed with 0.05 M Tris-buffered saline. In order to minimize subsequent loss of label, sections were post-fixed for 2 min in 2.5% glutaraldehyde and after extensive washing with double-distilled water, immunogold reactions were enhanced by incubating the sections for 5 to 10 min with the developing solution, freshly prepared prior to use (200 mg hydroquinone in 40 ml citrate buffer, pH=3.8 supplemented with 80 mg silver acetate in 40 ml double-distilled water). The development of the reactions was carried out in the dark in order to prevent non-specific precipitates of metallic silver. The reactions were stopped by washing the sections with 4% sodium thiosulfate for 5 min. After having been washed, the sections were dried at 40 °C, stained with toluidine blue and cover slipped. They were then examined under a Zeiss light microscope.

### Electron microscopy

Ultrathin sections (70 nm) were cut from different regions of the cirrhotic nodules and collected on formvar-coated nickel grids. Sections were incubated with ficin (24 mg/ml in double-distilled water) for 15 min at room temperature and then washed with double-distilled water. Unspecific binding sites were blocked with 1% bovine serum albumin in 0.05 M Tris-buffered saline for 10 min. Thereafter, ultrathin sections were processed for the immunohistochemical detection of laminins and of the integrin subunits α3 and α6. Immunohistochemistry was performed as described above, but primary antibodies (and secondary for laminin detection) were coupled to gold particles of 16nm instead of 8 nm. These were obtained by reducing 50 ml of 0.01% tetrachloroauric acid with 1 ml of 1% sodium citrate (De Mey et al., 1981; Slot and Geuze, 1981). After incubation with the antibody the sections were rinsed with 0.05 M Tris- buffered saline and stained with uranyl acetate for 10 min and lead citrate for 10 min at room temperature. They were then examined with a Zeiss EM 109 electron microscope.

### Control reactions

To exclude unspecific binding of colloidal gold particles, semithin and ultrathin sections were incubated with pure gold solution under the same conditions as described above. Additional negative controls were carried out by incubating sections with a gold-labeled anti-rabbit antibody (Medac, Hamburg, Germany).

## Results

### Ultrastructure

Parenchymal microvessels of cirrhotic liver were lined with a continuous endothelium. Microvilli of hepatocytes were bent along the hepatocytic surface. A basement membrane positive for tannic acid (Figs. 1-3), was visible between endothelial cells and hepatocytes. The basement membrane of the capillarized sinusoids showed a clear lamina densa in close proximity to its endothelial side. Between this lamina densa and the membrane of the endothelial cells a thin lamina rara was visible. A further higher density of the tannic acid-positive material was visible near the hepatocytes, forming another lamina densa. This was separated from the hepatocytes by a narrow and irregular lamina rara. In the space between the two laminae densae filamentous basement membrane material strongly positive for tannic acid was found.

### Immunohistochemistry

Laminin localization

After incubation of the semithin sections with anti-laminin antibodies (Fig. 4a), a clear continuous staining lining the parenchymal microvessels was seen. At the level of the basement membrane zones of bile ducts and the stromal blood vessels, staining was also seen. At the ultrastructural level (Fig. 4b), gold labeling for laminin was diffusely distributed throughout the basement

membrane of capillarized sinusoids, as well as in other basement membranes (e.g. bile ducts).

Alpha-3 integrin localization

After incubation of the semithin sections of cirrhotic human liver with anti-α3 antibodies (Fig. 5a), a clear continuous staining lining the capillarized sinusoids was observed. The basement membrane zones of bile ducts and of stromal blood vessels located within fibrotic septa were also clearly stained. Moreover, there was weaker staining in mesenchymal cells located in the fibrotic septa.

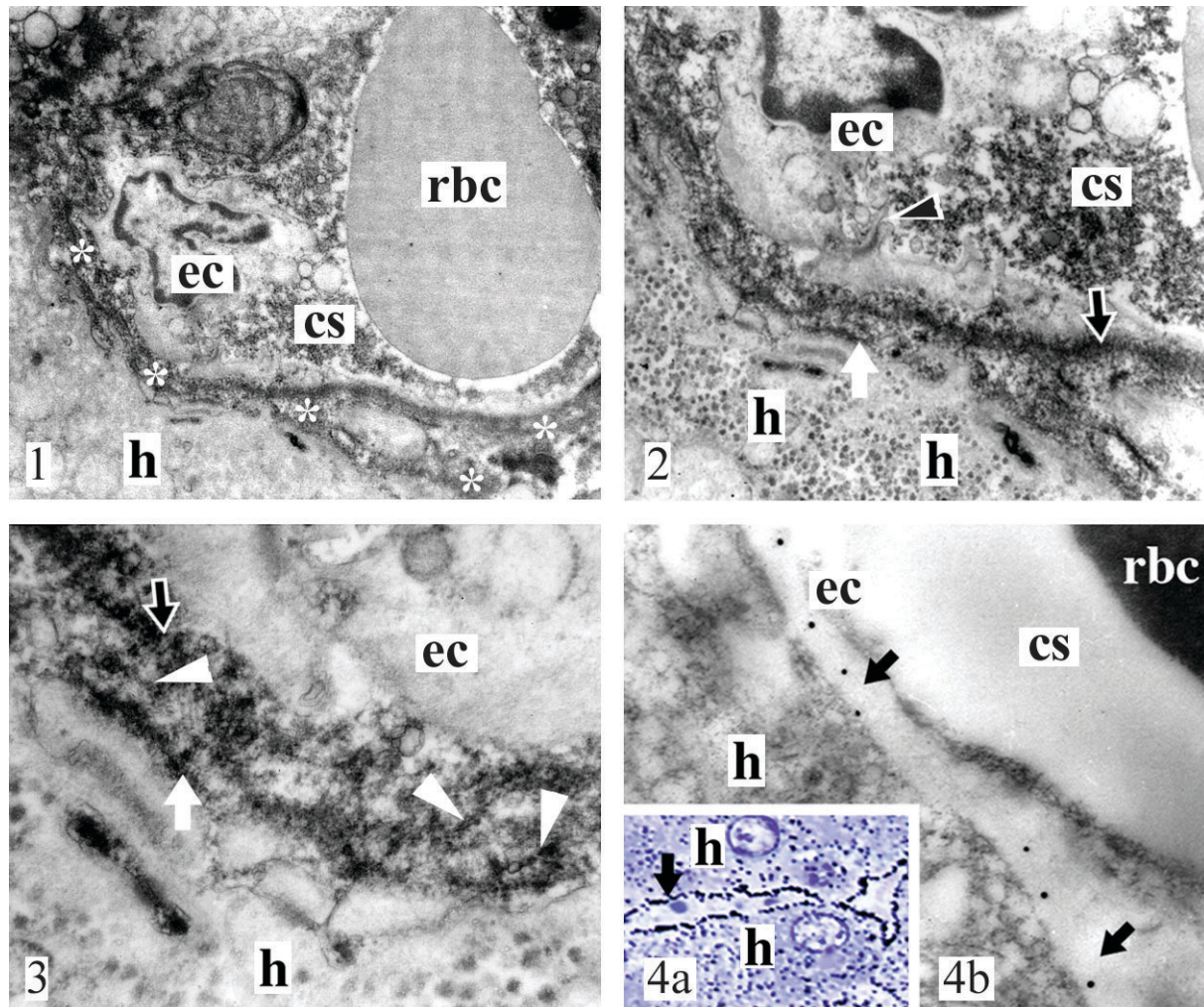At the ultrastructural level, human cirrhotic



**Fig. 1.** Cirrhotic human liver after fixation with addition of tannic acid. A continuous endothelium lines the capillarized sinusoid (cs) in which a red blood cell (rbc) is visible. A tannic acid-positive basement membrane (white asterisks) separates the endothelial cells (ec) from hepatocytes (h). x 3500

**Fig. 2.** Cirrhotic human liver after fixation with addition of tannic acid (higher magnification of the previous figure). A continuous endothelium lines the capillariezed sinusoid (cs). The black/white arrowhead points to a tight junction between endothelial cells (ec). A structured basement membrane positive to tannic acid is visible in the space of Disse between hepatocytes (h) and endothelial cells (ec). A higher density of the tannic acid-positive material is visible in the proximity of endothelial cells (black/white arrow) and of hepatocytes (white arrow) forming laminae densae. x 7500

**Fig. 3.** Higher magnification of the basement membrane depicted in Fig 1 and 2. Note the laminae densae at the hepatocytic side (white arrow) and at the endothelial side (black/white arrow) as well as the filamentous material of the basement membrane which fills the space of Disse (arrowheads). h= hepatocyte; ec: endothelial cell. x 18000

**Fig. 4. a.** Postembedding immunogold for laminin detection on a semithin section of cirrhotic human liver parenchyme. Capillarized sinusoids are lined by positive immunostaining (arrows). h: hepatocytes. x 1000. **b.** Cirrhotic human liver. Laminin detection in capillarized sinusoids by means of ultrastructural postembedding immunogold. Positive staining (arrows) is present between hepatocytes (h) and endothelial cells (ec). cs: lumen of the capillarized sinusoid; rbc: red blood cell. x 11000
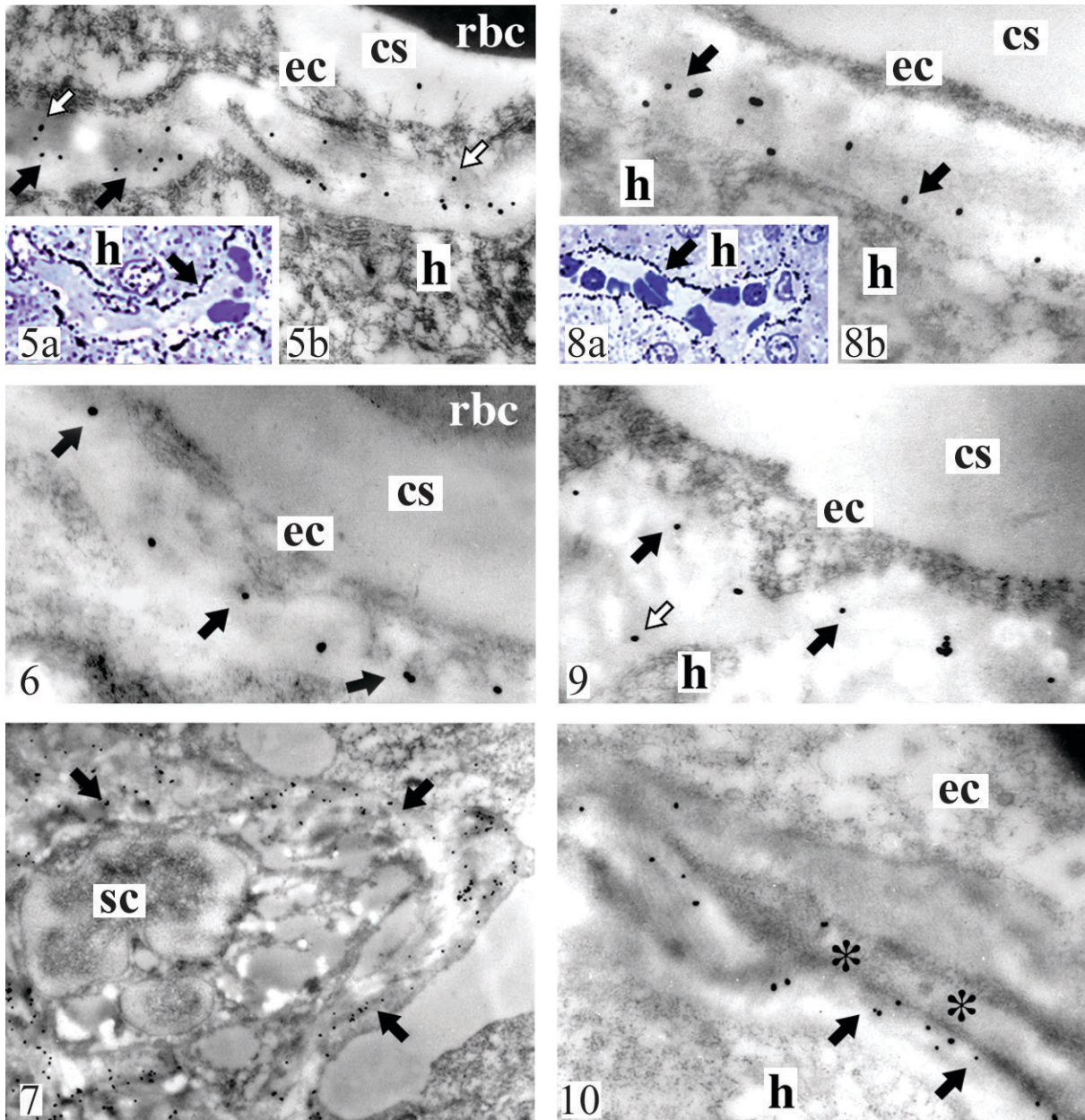
**Fig. 5. a.** Postembedding immunogold for the detection of the $\alpha3$ integrin subunit on a semithin section of cirrhotic human liver parenchyme. Capillarized sinusoids are lined by positive immunostaining (arrows). h= hepatocytes. x 1000. **b.** Cirrhotic human liver. Ultrastructural localization of the $\alpha3$ integrin subunit by means of postembedding immunogold in capillarized sinusoids. Positive staining is present both on hepatocytic (black arrows) and on the endothelial (open arrows) surfaces. h: hepatocytes; ec: endothelial cell; cs: lumen of the capillarized sinusoid; rbc: red blood cell. x 11000

**Fig. 6.** Cirrhotic human liver. Ultrastructural localization of the $\alpha3$ integrin subunit by means of postembedding immunogold in capillarized sinusoids. Positive staining (arrows) is present at the surface of endothelial cells (ec). cs: lumen of the capillarized sinusoid; rbc: red blood cell. x 15000.

**Fig. 7.** Stellate cell (sc) of cirrhotic human liver after ultrastructural localization of the $\alpha3$ integrin subunit. Positive staining (arrows) is present on the stellate cell (sc) surface. x 5250

**Fig. 8. a.** Postembedding immunogold for the detection of the $\alpha6$ integrin subunit on a semithin section of cirrhotic human liver parenchyme. Capillarized sinusoids are lined by positive immunostaining (arrows). h: hepatocytes. x 1000. **b.** Cirrhotic human liver. Ultrastructural localization of the $\alpha6$ integrin subunit by means of postembedding immunogold in capillarized sinusoids. Positive staining is present on the hepatocytic (black arrows) surface. h: hepatocytes; ec: endothelial cell; cs: lumen of the capillarized sinusoid. x 15000

**Fig. 9.** Cirrhotic human liver. Ultrastructural localization of the $\alpha6$ integrin subunit by means of postembedding immunogold in capillarized sinusoids. Positive staining is present on endothelial (black arrows) and on hepatocytic (open arrow) surfaces. h: hepatocytes; ec: endothelial cell; cs: lumen of the capillarized sinusoid. x 15000

**Fig. 10.** Stellate cell prolongation (**) of cirrhotic human liver after ultrastructural localization of the $\alpha6$ integrin subunit. Positive staining (black arrows) is present along the stellate cell surface. h: hepatocyte; ec: endothelial cell. x 13000

parenchymal microvessels showed staining for α3-integrin which was located along the extracytoplasmic side of the hepatocytic cell surface (Fig. 5b), as well as over the abluminal cell surface of the microvascular endothelium (Fig. 6). The pattern was often focal. The surfaces of stellate cells were also stained (Fig. 7).

Alpha-6 integrin localization.

After incubation of the semithin sections with anti-α6 antibodies (Fig. 8a) the pattern of the immunohistochemical staining did not differ from that of α3 staining. Also, in the case of α6, staining was seen to line intraparenchymal microvessels as well as the basement membrane zones of all bile ducts and stromal blood vessels. Staining was also seen on mesenchymal cells located in the fibrotic septa.

Ultrastructurally, in the perisinusoidal space of human capillarized sinusoids gold labeling was observed along the extracytoplasmic side of the hepatocytic (Fig. 8b) as well as of the endothelial cell surfaces (Fig. 9). Labeling for α6 also occurred in a focal pattern, similar to that of α3. Gold labeling was also detected on stellate cell surfaces (Fig. 10).

## Discussion

In the present work we have shown for the first time the ultrastructural distribution of integrin subunits α3 and α6 in capillarized sinusoids of cirrhotic human liver. The distribution of such integrin subunits in human liver cirrhosis has previously been studied at the light microscopical level (Volpes et al., 1991; Couvelard et al., 1993; Torimura et al., 1997). In contrast, their cellular distribution has never been shown at the ultrastructural level. However, due to the the limited resolution of light microscopy, only an ultrastructural investigation would clarify the cellular distribution of such integrin subunits in cirrhotic liver sinusoids. We aimed to answer this question and, therefore, localized α3 and α6 integrin subunits ultrastructurally.

Here we have shown that in capillarized sinusoids of the cirrhotic human liver, α3 and α6 integrin subunits are located on the surface of endothelial cells, of hepatocytes and of stellate cells. The integrin subunit α3 was reported to be mainly located at the periphery of the cirrhotic nodules in frozen sections (Couvelard et al., 1993) but we did not note any particular topographic correlation. Probably, the specimens that we examined were of later stages and in the later stages of cirrhosis, α3 integrin molecules in the liver may increase. This is conceivable since α3ß1 integrins are essential for basement membrane structure (Kreidberg et al., 1996; Di Persio et al., 1997) and basement membrane formation is a late event in cirrhosis, as reported from experimental $CCl_4$-induced cirrhosis (Martinez-Hernandez and Amenta, 1993). This could also explain discrepancies with the results of Torimura et al. (1997) who, in contrast to us and to Couvelard et al. (1993), did not find any α3 integrin in cirrhotic livers at all.

It was striking, that the gold staining of integrins seems to appear at some distance from the cell surfaces and projects into the extracellular space. This may primarily be due to the omission of postfixation of the tissue with osmium tetroxide as required by the immunohistochemical technique used. This leads to a poor preservation of the cell membranes and therefore reduces the definition of the cell limits. Moreover, the antibodies used (J143 for α3 and GoH3 for α6) are directed against the extracellular domains of the corresponding integrin subunits.

Since integrins are dimers (Hynes, 1992; van der Flier and Sonnenberg, 2001), one question is which of the known integrins are localized by the immunohistochemical detection of α3 and α6 subunits in human cirrhotic liver. The subunit α3 associates only with ß1 to form α3ß1 (Hynes, 1992; van der Flier and Sonnenberg, 2001), therefore its detection relates to integrin α3ß1. In contrast, α6 can associate both with ß1 and ß4 (Hynes, 1992; van der Flier and Sonnenberg, 2001). However, there is evidence that ß4 subunit occurs in smaller amounts than ß1 in liver cirrhosis (Couvelard et al., 1993). Therefore, one can assume that the immunohistochemical detection of α6 subunit in human cirrhotic liver nodules mainly relates to the integrin α6ß1, even though α6b4 cannot completely be excluded. Integrins of the ß1-class play an important role for basement membrane formation and matrix production (Kreidberg et al., 1996; DiPersio et al., 1997; Sasaki et al., 1998; Aumailley et al., 2000; Brakebusch et al., 2000; Raghavan et al., 2000; Li et al., 2002). Therefore, the elucidation of the cellular distribution of α3 and α6 in sinusoids of the cirrhotic human liver (and therefore of α3ß1 and α6ß1) would be help to clarify which cells may contribute to the process of capillarization of the sinusoids, which is a key event in cirrhosis (Schaffner and Popper, 1963; Martinez-Hernandez and Martinez, 1991).

As shown here, in the capillarized sinusoids of human liver cirrhosis, integrin subunits α3 and α6 are present along both the membranes of hepatocytes and endothelial cells. This indicates that α3ß1 and α6ß1 are expressed both in hepatocytes and endothelial cells. This correlates with sinusoidal capillarization. In fact, we also show laminin molecules in the perisinusoidal space and presence of a basement membrane which are known markers of sinusoidal capillarization (Schaffner and Popper, 1963; Hahn et al., 1980).

In the newly formed pathological perisinusoidal basement membrane, structures which resemble laminae densae can be found both in the proximity of the hepatocytic and of the endothelial cell borders. This, together with laminin detection in the perisinusoidal space and with localization of α3ß1 and α6ß1 in hepatocytes and endothelial cells, indicates that in sinusoids of cirrhotic human liver the integrins α3ß1 and α6ß1 are expressed on both sides of the newly formed pathological basement membrane. One interesting

question is what the influence of these integrins on hepatocytes and on endothelial cells in the human cirrhotic liver may be.

It is possible that these ß1-class integrins may somehow contribute to the organization of the newly formed pathological basement membrane which completely fills the space of Disse and separates the capillarized sinusoids from the hepatocytes. There is in fact much evidence that indicates that ß1-class integrins are essential for the formation of a structured and stabilised basement membrane (Kreidberg et al., 1996; DiPersio et al., 1997; Sasaki et al., 1998; Aumailley et al., 2000; Brakebusch et al., 2000; Raghavan et al., 2000; Li et al., 2002). Also, evidence that argues in favour of ß1-class integrins as organisational factors for basement membrane structures at the cell surface has been provided (Fleischmajer et al., 1998a,b; Henry et al., 2001; Lohikagans et al., 2001). According to this view, ß1-class integrins (possibly in concert with other receptors, e.g. dystroglycan or further, still unknown molecules) could catalyse the self-assembly of basement membrane components and stabilise the basement membranes formed. Our in vivo data from human liver cirrhosis, showing integrins α3ß1 and α6ß1, i.e. receptors for basement membrane components, at locations adjacent to the newly formed pathological basement membrane of sinusoids are consistent with this view.

It is also known that signalling mediated by ß1-class integrins may also modulate the production of basement membrane components (Sasaki et al., 1998; Aumailley et al., 2000; Li et al., 2002). In the present work, we have demonstrated the presence of α3 and α6 integrin subunits in stellate cells in vivo for the first time. In this context, one can assume that the presence of α3ß1, α6ß1 (and possibly α6ß4) integrins on stellate cells may regulate a signalling pathway necessary for basement membrane production. Stellate cells are the main producers of extracellular matrix molecules and thus also of basement membrane components in liver cirrhosis (Reeves and Friedman, 2002). It has been shown that the integrin subunit α6 is expressed by stellate cells in culture (Carloni et al., 1996). Here we show this in vivo and we also show that stellate cells in vivo express the subunit α3.

In summary, in the present work, we have shown the ultrastructural distribution of integrin subunits α3 and α6 in cirrhotic human capillarized liver sinusoids for the first time on the surface of endothelial cells, of hepatocytes and of stellate cells. Our findings indicate that, *in vivo*, the expression of integrins α3ß1 and α6ß1 (and probably also α6ß4) correlate with basement membrane formation in capillarized human cirrhotic sinusoids. This suggests that the notion that ß1-integrins take part in regulation of basement membrane formation may apply to the formation of the perisinusoidal basement membrane in human cirrhotic liver as well. In line with this, our findings also suggest that the suppression of the expression of such integrins could be

a possible therapeutic approach towards inhibiting basement membrane formation in the cirrhotic liver in the future. This could contribute to an improvement of the metabolic function of the liver in the course of hepatic cirrhosis.

## References

Aumailley M., Pesch M., Tungaal L., Gaill F. and Fässler R. (2000). Altered synthesis of laminin-1 and absence of basement membrane component deposition in ß1-integrin deficient embryoid bodies. J. Cell Sci. 113, 259-268.

Brakebusch C., Grose R., Quondamatteo F., Ramirez A., Pirro A., Svensson M., Herken R., Sasaki T., Timpl R., Werner S. and Fässler R. (2000). Skin and hair follicle integrity is crucially dependent on ß1 integrin expression on keratinocytes. EMBO J. 19, 3990-4003.

Burgeson R.E., Chiquet M., Deutzmann R., Ekblom P., Engel J., Kleinmann H., Martin G.R., Meneguzzi G., Paulsson M., Sanes J., Timpl R., Trygvasson K., Yamada Y. and Yurchenco P.D. (1994). A new nomenclature for the laminins. Matrix Biol. 1994 14, 209-211.

Carloni V., Romanelli R.G., Pinzani M., Laffi G. and Gentilini P. (1996). Expression and function of human integrin receptors for collagen and laminin in cultured human hepatic stellate cells. Gastroenterology 110, 1127-1136.

Couvelard A., Scoazec J.Y. and Feldmann G. (1993). Expression of cell-cell and cell-matrix adhesion proteins by sinusoidal endothelial cells in the normal and cirrhotic human liver. Am. J. Pathol. 143, 738-752

De Mey J., Moersmans N., Geuens G., Nydens R. and De Brabander M. (1981). High resulution light and electron microscopic localization of tubulin with IGS (immuno gold staining) method. Cell Biol. Int. 5, 889-899.

Di Persio C.M., Hodivala-Dilke K.M., Jaenisch R., Kreidberg J.A. and Hynes R.O. (1997). α3ß1 integrin is required for normal development of the epidermal basement membrane. J. Cell Biol. 137, 729-742

Fleischmajer R., Perlish J.S., Macdonald II E.D., Schechter A., Murdoch A.D., Iozzo R.V. and Yamada Y. (1998a). There is binding of collagen IV to ß1 integrin during early skin basement membrane assembly. Ann. N.Y. Acad. Sci. 857, 212-227.

Fleischmajer R., Utani A., Macdonald II E.D., Perlish J.S., Pan T.C., Chu M.L., Nomizu M., Ninomiya Y. and Yamada Y. (1998b). Initiation of basement membrane formation at the epidemo-dermal interface involves assembly of laminins through binding to cell membrane receptors. J. Cell Sci. 111, 1929-1940.

Hahn E., Wick G., Pencev D. and Timpl R. (1980). Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin and fibronectin. Gut 21, 63-71.

Henry M.D., Satz J., Brakebusch C., Costell M., Gustafsson E., Fässler R. and Campbell K.P. (2001). Distinct roles for dystroglycan, ß1-integrin and perlecan in cell surface laminin organization. J. Cell Sci. 114, 1137-1144.

Herken R. and Barrach H.-J. (1985). Ultrastructural localization of type IV collagen and laminin in the seven-day-old mouse embryo. Anat.

Embryol. 171, 365-371.

Herken R. and Miosge N. (1991). Postembedding immunogold histochemistry for the localization of laminin and its E4 and P1 fragments in the mouse kidney embedded in LR-White and LR-Gold. Histochem. J. 23, 267-272

Herken R., Manshausen B., Fussek M. and Bonatz G. (1987). Methodological dependence in the ultrastructural immunolocalization of laminin in tubular basement membranes of the mouse kidney. Histochem. Cell Biol. 87, 59-64.

Hynes R.O. (1992). Integrins: versatility; modulation, and signalling in cell adhesion. Cell 69, 11-25.

Kantor R.R., Mattes M.J., Lloyd K.O., Old L.J. and Albino A.P. (1987). Biochemical analysis of two cell surface glycoprotein complexes, very common antigen 1 and very common antigen 2. Relationship to very late activation T cell antigens. J. Biol. Chem. 262, 15158-15165.

Kreidberg J.A., Donovan M.J., Goldstein S.L., Rennke H., Shepherd K., Jones R.C. and Jaenisch R. (1996). Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. Development 122, 3537-3547

Li S., Harrison D., Carbonetto S., Fässler R., Smyth N., Edgar D. and Yurchenco P.D. (2002). Matrix assembly, regulation and survival functions of laminin and its receptors in embryonic stem cell differentiation. J. Cell Biol. 157, 1279-1290.

Lohikangas L., Gullberg D. and Johansson S. (2001). Assembly of laminin polymers is dependent on ß1-integrins. Exp. Cell Res. 265, 135-144..

Martinez-Hernandez A. and Amenta P.S. (1993). The hepatic extracellular matrix. II. Ontogenesis, regeneration and cirrhosis. Virchows Arch. (A) 423, 77-84.

Martinez-Hernandez A. and Martinez J. (1991). The role of capillarization in hepatic failure: studies in carbon tetrachloride-induced cirrhosis. Hepatology 14, 864-874

Murdoch A.D., Liu B., Schwarting R., Tuan R.S. and Iozzo R.V. (1994). Widespread expression of perlecan proteoglycan in basement membranes and extracellular matrices of human tissues as detected by a novel monoclonal antibody against domain III and by in situ hybridization. J. Histochem. Cytochem. 42, 239-249.

Quondamatteo F., Michalski S., Wesling F., Schlemminger R., Miosge N. and Herken R. (1998). Laminin localization in the enterocytic basement membrane of small bowel grafts. A light and electron microscopic study. Matrix Biol. 17, 647-655.

Raghavan S., Bauer C., Mundschau G., Li Q. and Fuchs E. (2000). Conditional ablation of ß1 integrin in skin: severe defects in epidermal proliferation, basement membrane formation and hair follicle invagination. J. Cell Biol. 150, 1149-1160.

Reeves H.L. and Friedman S.L. (2002). Activation of hepatic stellate cells- a key issue in liver fibrosis. Front. Biosci. 7, d808-826.

Sasaki T., Forsberg E., Bloch W., Addicks K., Fässler R. and Timpl R. (1998). Deficiency of ß1 integrins in teratoma interferes with basement membrane assembly and laminin-1 expression. Exp. Cell Res. 238, 70-81.

Schaffner F. and Popper H. (1963). Capillarization of hepatic sinusoids in man. Gastroenterology 44, 239-242.

Scoazec J.Y. (1995). Expression of cell-matrix adhesion molecules in the liver and their modulation during fibrosis. J. Hepatol. 22, 20-27.

Slot J.W. and Geuze H.J. (1981). Sizing of protein A-colloidal gold probes for immunoelectron microscopy. J. Cell Biol. 90, 533-536.

Sonnenberg A., Janssen H., Hogervorst F., Calafat J. and Hilgers J. (1987). A complex of glycoproteins Ic and IIa identified by a rat monoclonal antibody. J. Biol. Chem. 262, 10376-10383.

Torimura T., Ueno T., Kin M., Inuzuka S., Sugawara H., Tamaki S., Tsuji R., Sujaku K., Sata M. and Tanikawa K. (1997). Coordinated expression of integrin α6ß1 and laminin in hepatocellular carcinoma. Hum. Pathol. 28, 1131-1138.

Yurchenco P.D. (1994). Assembly of laminin and type IV collagen into basement membrane networks. In: Extracellular matrix assembly and structure. Yurchenco P.D., Birk D.E. and Mecham R.P. (eds). Academic Press. San Diego. pp 351-388.

van der Flier A. and Sonnenberg A. (2001). Function and interactions of integrins. Cell Tissue Res. 305, 285-298.

Volpes R., van den Oord J.J. and Desmet V. (1991). Distribution of the VLA family of integrins in normal and pathological human liver tissue. Gastroenterology 101, 200-206.