

# Role of $\alpha_v$ integrins in mesangial cell adhesion to vitronectin and von Willebrand factor

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**Role of  $\alpha_v$  integrins in mesangial cells adhesion to vitronectin and von Willebrand factor.** This study demonstrates (by flow cytometry and immunoprecipitation after cell surface radiolabeling and by using monoclonal antibodies to  $\alpha_v$ ,  $\beta_3$ , and  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  complexes) that  $\alpha_v\beta_3$ , the vitronectin receptor, and  $\alpha_v\beta_5$  are expressed *in vitro* on cultured human mesangial cells (HMC) of the 5th to 8th passages. Antibodies to  $\alpha_v$ ,  $\beta_3$  and  $\alpha_v\beta_3$  respectively precipitated an  $\alpha\beta$  heterodimer with molecular weights of 140 and 97 kDa. We analyzed the role of the various integrins in HMC interactions with vitronectin, and with fibronectin and von Willebrand factor (vWf), which are synthesized respectively by mesangial and endothelial cells. Cell adhesion increased in a dose dependent manner with the concentration of plastic-coated matrix protein and vWf. Inhibition of cell attachment with monoclonal antibodies to integrins indicated that HMC adhesion to vWf primarily involves  $\alpha_v\beta_3$ , and that  $\alpha_v\beta_5$  may also contribute to cell binding to vWf. Adhesion to vitronectin involves both  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  complexes. In contrast, adhesion to fibronectin was not affected by monoclonal antibodies to  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  complexes. We propose that integrins  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ , present on HMC, could mediate an interaction between mesangial and endothelial cells by binding to vWf, released at the basal site of endothelial cells.

Integrins are heterodimeric transmembrane glycoproteins consisting of varying combinations of noncovalently bound  $\alpha$  and  $\beta$  chains, and are involved in adhesive interactions with the extracellular matrix. They are critical for normal physiological processes such as embryonic development, tissue structure and wound repair [1].

Integrins that contain the  $\alpha_v$  chain mediate attachment of a variety of cells to diverse matrix proteins [2] and are critical for angiogenesis [3, 4], migration [5], cell proliferation and survival [6–8]. The integrin  $\alpha_v\beta_3$ , the vitronectin receptor, is the most promiscuous member of the integrin family, allowing cells to interact with a wide variety of proteins including vitronectin, fibrinogen, von Willebrand factor, thrombospondin, fibronectin, osteopontin, laminin and denatured collagen types I and IV [2].  $\alpha_v\beta_3$  is expressed on a variety of cells including endothelial cells, fibroblasts, melanoma, osteosarcoma and smooth muscle cells. In addition to  $\beta_3$ ,  $\alpha_v$  chain associates with multiple  $\beta$  chains, that is,

$\beta_1$ ,  $\beta_5$ ,  $\beta_6$  and  $\beta_8$ . The nature of cellular interactions with matrix proteins apparently depends on the  $\beta$  subunit.  $\alpha_v\beta_1$  and  $\alpha_v\beta_5$  recognize fibronectin and vitronectin [9], while  $\alpha_v\beta_6$  binds to fibronectin [10, 11] and  $\alpha_v\beta_8$  binds to vitronectin [12].

The renal localization of  $\beta_3$  integrins has been investigated in developing and adult kidneys [13–15]. In the human adult glomerulus,  $\alpha_v\beta_3$  is normally strongly expressed by glomerular endothelial cells, capsular epithelial cells and podocytes, and to a lesser extent by mesangial cells [13–18]. In the developing glomeruli, the  $\beta_3$  chain is expressed in the cells of Bowman's capsule [19]. The role of  $\alpha_v\beta_3$  in the metanephric development is suggested by its spatio-temporal distribution that varies with time, is restricted to the epithelia before vascularization, and is concentrated in the glomerular and extraglomerular vasculature in the mature kidney [19].

In the present study, we investigated the presence and the adhesive function of the  $\alpha_v$  family of integrins on human mesangial cells in culture. We present evidence that  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  and possibly  $\alpha_v\beta_1$  integrins are expressed at the membrane of this cell type. We also show that adhesion of cultured HMC to vitronectin and to vWf involves  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ .

## Methods

### Antibodies

The antibodies used in this study are presented in Table 1.

Monoclonal antibodies included: anti- $\alpha_v$  (clone AMF/7), anti- $\alpha_6$  (clone GoH3), anti- $\alpha_2$  (clone Gi9), anti- $\beta_1$  (clone Lia 1/2) and control mouse IgG were from Immunotech (Marseille, France); anti- $\alpha_3$  (clone P1B5) was from Chemicon (Temecula, CA, USA); anti- $\alpha_5$  (clone 11, 16) was a generous gift from Dr. Steven K. Akiyama (Laboratory of Developmental Biology, NIH, MD, USA); anti- $\alpha_v$  (clone LM142, ascites) and anti- $\alpha_v\beta_3$  (clone LM 609, ascites) were a generous gift from Dr. David Chersesh (Scripps Research Institute, La Jolla, CA, USA); anti- $\beta_3$  (clone AP3, ascites) was a generous gift from Dr. Peter J. Newman (The Blood Center of Southeastern, WI, USA); anti- $\beta_1$  (clone K20) was a generous gift from Dr. Alain Bernard (INSERM U 343, Nice, France); anti- $\alpha_v\beta_5$  (clone P1F6) was from Chemicon; FITC-goat anti-mouse F(ab')<sub>2</sub> IgG fragments were from Jackson IRL (West Grove, PA, USA). The concentration for maximal monoclonal antibody binding was determined individually by flow cytometry and excess concentrations were used, as indicated,

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**Table 1.** Antibodies to integrins used in this study

Specificity	Clone	Species	Reference
$\alpha_2$	Gi9	mouse	[20]
$\alpha_3$	P1B5 <sup>a</sup>	mouse	[21]
$\alpha_5$	11	mouse	[22]
	16 <sup>a</sup>	mouse	
$\alpha_6$	GoH3 <sup>a</sup>	rat	[23]
$\alpha_v$	AMF7	mouse	[24]
	LM142	mouse	[25]
	Lia1/2 <sup>a</sup>	mouse	[26]
$\beta_1$	K20	mouse	[27]
$\beta_3$	AP3	mouse	[28]
$\beta_5$	polyclonal	rabbit	[29]
$\alpha_v\beta_3$	LM609 <sup>a</sup>	mouse	[25]
$\alpha_v\beta_5$	PIF6 <sup>a</sup>	mouse	[30]

<sup>a</sup>Antibodies known to inhibit cell adhesion. The origin of these antibodies is indicated in the text.

during incubation both for flow cytometry analysis and adhesion inhibition experiments. Rabbit polyclonal anti- $\beta_5$  antibodies were from Novus Molecular (San Diego, CA, USA). Biotinylated goat anti-mouse IgG and anti-rabbit IgG were respectively from Immunotech and from Novus Molecular.

#### Matrix components

Human vitronectin was purchased from Becton Dickinson (Bedford, MA, USA), human fibronectin from Sigma (St. Louis, MO, USA). According to the manufacturer, both proteins were purified to homogeneity (> 95%) as assessed by SDS PAGE analysis. Von Willebrand factor (vWf) was purified to homogeneity as described [31]. Purified vWf was homogeneous (> 95%) and vitronectin was not detectable by SDS PAGE analysis.

#### Human mesangial cells

Human mesangial cells (HMC) were prepared from the normal pole of kidney obtained after nephrectomy as previously described [32]. Briefly, each kidney was decapsulated, glomeruli were isolated from renal cortex by serial sieving, treated with collagenase type IV 300 UI/ml (Sigma) 20 to 30 minutes at 37°C, washed, and cultured in flasks with RPMI 1640 (ATGC, Paris, France) supplemented with 10% heat-inactivated fetal bovine serum FBS (ATGC), 2 mM L-glutamin, 60 UI/ml penicillin, 60 UI/ml streptomycin, and 10 mM HEPES at 37°C under 5% CO<sub>2</sub>. HMC grown from glomeruli within two to three weeks were then subcultured and maintained in 10% FBS/RPMI medium. HMC were shown to be free of mycoplasma and characterized by morphology,  $\alpha$  smooth muscle actin expression, absence of factor VIII expression, and used at the 5th to 8th passages.

#### Human endothelial cells

Human umbilical vein endothelial cells (HUVEC) were isolated as previously described by Jaffe et al [33]. HUVEC were cultured in M-199 (ATGC, Paris, France) supplemented with 20% heat-inactivated FBS (ATGC), 2 mM L-glutamine, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, 0.25  $\mu$ g/ml amphotericin B, 10 mM HEPES, 50  $\mu$ g/ml heparin, 20  $\mu$ g/ml endothelial cell growth factor at 37°C under 5% CO<sub>2</sub>, and used after two passages.

#### Fibroblasts

Fibroblasts were obtained from forearm skin biopsies as described [34] and grown in the same condition as we used for mesangial cell culture (see above) with 20% heat-inactivated FBS.

#### Flow cytometry

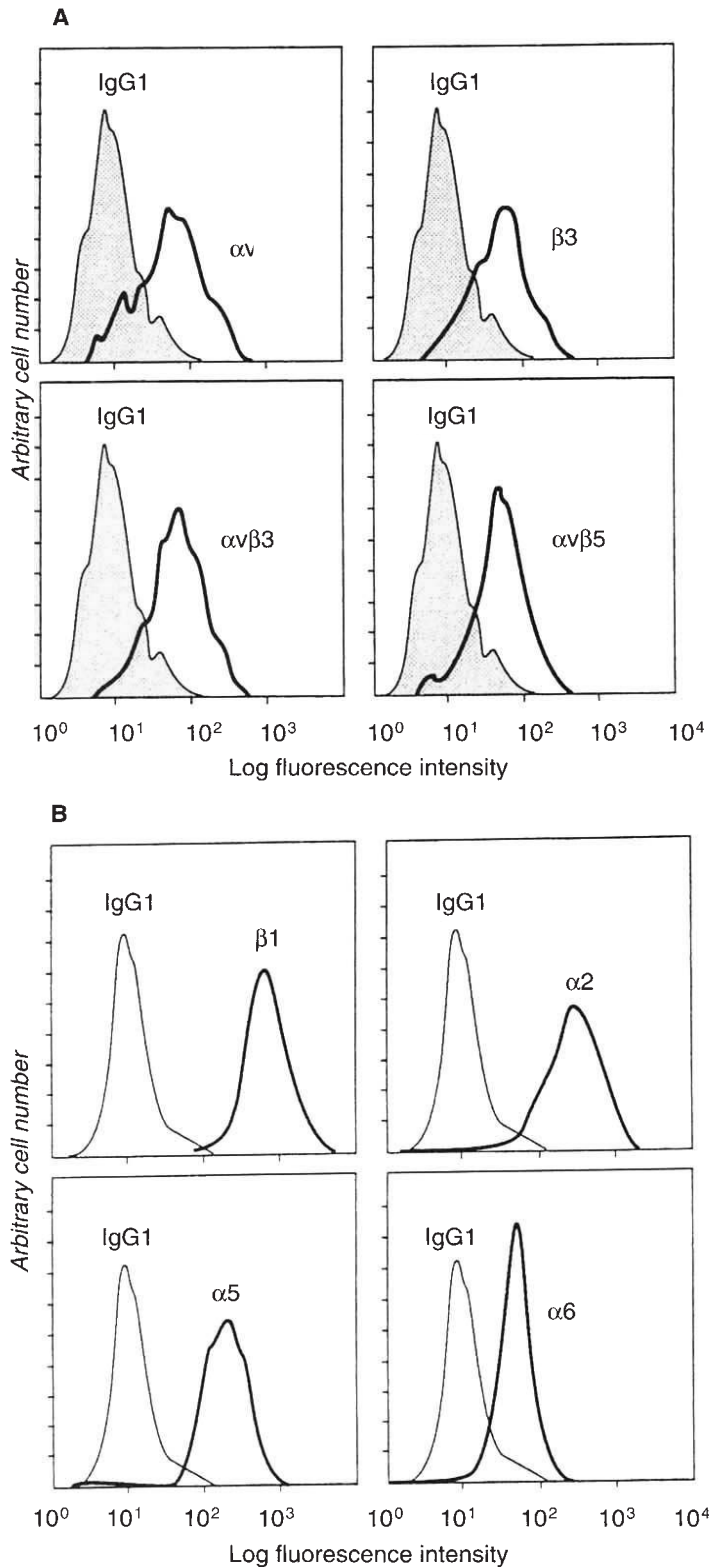
Expression of integrins by cultured cells in suspension was evaluated by flow cytometry. HMC were detached from culture flasks by trypsin (ATGC) treatment for five minutes at 37°C, washed with RPMI, counted and resuspended in PBS containing 1% BSA and 0.1% sodium azide. A total of 10<sup>5</sup> cells were incubated at 4°C with anti-integrin antibodies for 30 minutes, washed three times with PBS and stained with fluorescein-conjugated goat anti-mouse IgG. Stained cells were fixed with 1% formaldehyde and analyzed on a FACScan flow cytometer (Becton Dickinson).

#### Adhesion assays

Adhesion assays were performed in 96-well plates (Costar, Cambridge, MA, USA) coated with 50  $\mu$ l/well of matrix proteins in PBS: fibronectin (1  $\mu$ g/ml), vitronectin (0.5  $\mu$ g/ml), vWf (5  $\mu$ g/ml). Plates were incubated at 37°C for 60 minutes, washed three times with phosphate-buffered saline (PBS) and unbound sites were blocked one hour at 37°C with 1% BSA (Sigma) previously heat-inactivated for 60 minutes at 56°C. Control wells were coated with 1% heat-inactivated BSA. HMC were detached from culture flasks by trypsin/EDTA treatment, and resuspended in RPMI with 0.1% BSA. A total of 10<sup>4</sup> cells were allowed to adhere in each well at 37°C for 60 minutes. Non-adherent cells were then removed by washing with PBS, and adherent cells were fixed with 4% paraformaldehyde for five minutes. After rinsing with PBS, cells were stained for five minutes with 0.5% toluidine blue in 4% formaldehyde and rinsed with water. Cells were solubilized with 1% SDS and quantified in a microtiter plate reader at 630 nm. This procedure of quantitation gives values proportional to the number of adherent cells [24]. In adhesion inhibition experiments, cells were first preincubated for 30 minutes at 4°C with anti-integrin antibodies and treated thereafter as described above. All inhibition experiments were repeated independently, the number of which is indicated in each case.

#### Immunoprecipitation

Immunoprecipitation was performed on HMC. Cells were washed three times with PBS and surface labeled with 2 mCi/ml of Na<sup>125</sup>I in tubes coated with iodogen (Pierce, Rockford, IL, USA), according to the manufacturer's instructions. After 30 minutes at 4°C, cells were washed and solubilized with 2% NP-40 in PBS containing protease inhibitors: 2 mM PMSF, 1 mg/ml SBTI, 1000 U/ml aprotinin, 5 mM iodoacetamide and 1  $\mu$ g/ml leupeptin. The cell lysate was cleared by centrifugation for 10 minutes at 4°C and the supernatant, precleared on normal rabbit IgG protein A-Sepharose, was incubated with different antibodies coupled to protein A-Sepharose for three hours at 4°C on a roller. Antigen-antibody complexes bound to the Sepharose beads were pelleted on a microcentrifuge and washed ten times with PBS 0.1% NP40. Samples were analyzed by SDS-PAGE under nonreducing conditions on a 6% polyacrylamide gel and radiolabeled bands visualized

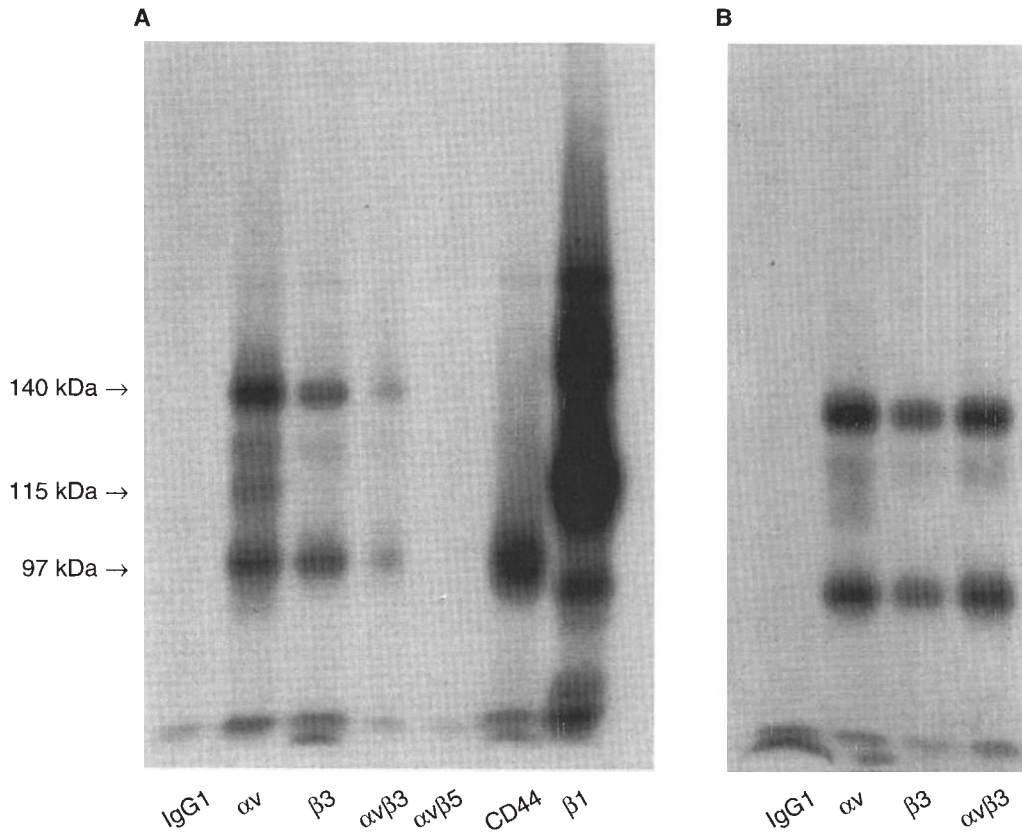


**Fig. 1.** FACS analysis of  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrins on mesangial cells. Suspended ( $10^5$ ) mesangial cells were labeled by indirect immunofluorescence and analyzed by flow cytometry, as described in the **Methods** section, with monoclonal antibodies to integrins: (A) anti- $\alpha_v$  (AMF/7 and LM142), anti- $\beta_3$  (AP3), anti- $\alpha_v\beta_3$  (LM609) and anti- $\alpha_v\beta_5$  (PIF6) antibodies; and (B) anti- $\alpha_2$  (Gi9), anti- $\alpha_5$  (16, 11), anti- $\alpha_6$  (GoH3) and anti- $\beta_1$  (K 20, Li1/2) antibodies. Cells were then stained with fluorescein-conjugated goat anti-mouse IgG (1:1000 dilution) and analyzed by flow cytometry. The ordinates depicts the number of cells per channel and the abscissa depicts the relative fluorescence intensity in arbitrary units (log scale). Control mouse monoclonal IgG was used as control. Representative experiment of six independent determinations.

by autoradiography. The molecular mass markers were myosine (200 kDa),  $\beta$ -galactosidase (116 kDa), phosphorylase-b (97 kDa), bovine serum albumin (66 kDa) and ovalbumin (45 kDa) (Biorad Lab., Paris, France).

#### Western blotting

HMC were solubilized with 2% NP40 in PBS with the same protease inhibitors as above for 30 minutes at 4°C. Proteins were



**Fig. 2.** Immunoprecipitation of  $\alpha_v$  integrins and CD44 by human mesangial and endothelial cells in culture. Subconfluent cultures of mesangial cells (A) and confluent cultures of endothelial cells (B) were surface radiolabeled, lysed with detergent and subjected to immunoprecipitation as described in the **Methods** section, with monoclonal antibodies directed to the  $\alpha_v$  chain (clone LM 142),  $\beta_3$  chain (clone AP3),  $\beta_1$  chain (K20) or to the complex  $\alpha_v\beta_3$  (LM 609) and  $\alpha_v\beta_5$  (P1F6) and to CD44 (J-173) used as control [50]. The precipitated material (HMC,  $10^6$  cells; HUVEC,  $4 \times 10^6$  cells) was analyzed by SDS-PAGE under non-reducing conditions and exposed to x-ray film as described. Representative gel of three independent immunoprecipitations.

analyzed by electrophoresis on 7.5% polyacrylamide gel under non-reducing conditions, and transferred to a nitrocellulose membrane by electrophoresis. After saturation overnight at room temperature with TBST (10 mM Tris-HCl, pH 8; 1 mM  $\text{NaN}_3$ ; 0.05% Tween 20; 0.15 M NaCl) containing 10% skimmed milk, membranes were incubated one hour at room temperature with anti-integrin  $\beta_3$  or anti- $\beta_5$  mAbs, followed by biotinylated anti-mouse or -rabbit IgG respectively, washed three times with TBST and incubated with HRP-streptavidin complex (Amersham, Buckinghamshire, UK). The membranes were finally revealed by chemiluminescence using an Amersham enhancing reagent according to the manufacturer's instruction.

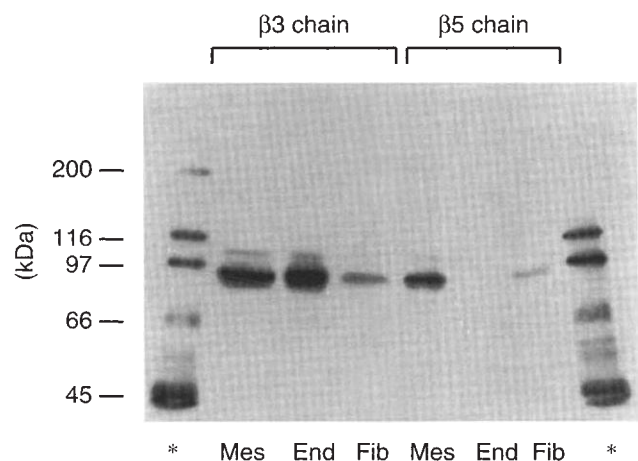
#### Statistical analysis

Results of cell adhesion inhibition assays were expressed as means  $\pm$  SEM of adherent cells (OD at 630 nm). Groups of duplicates were analyzed. Comparisons were made by ANOVA and post hoc comparisons were made by the Dunnett's test.

## Results

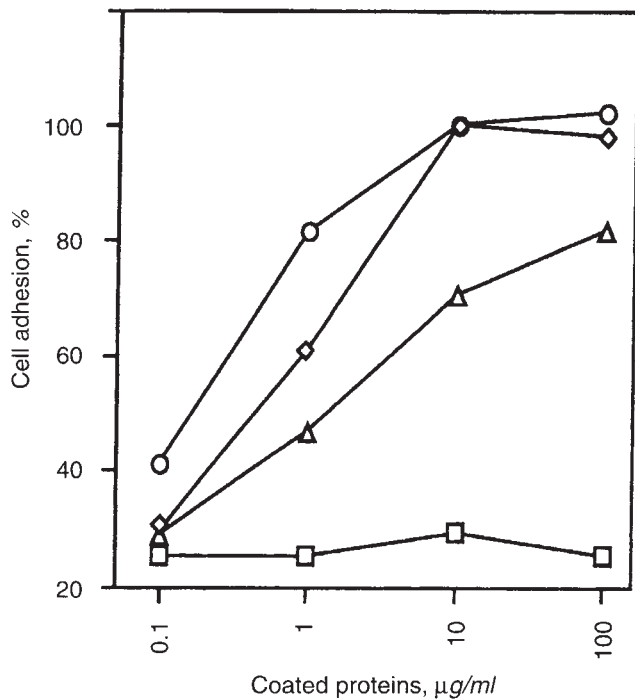
### Membrane expression

Surface expression of integrin  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  was examined by flow cytometry on suspended cells using monoclonal antibodies to



**Fig. 3.** Western blot analysis indicates that  $\beta_3$  and  $\beta_5$  chains are present on mesangial cells. HMC,  $10^6$  cells, (Mes); HUVEC,  $4 \times 10^6$  cells (End); human cultured fibroblasts,  $10^6$  cells (Fib) were solubilized and the blots were probed with antibodies to  $\beta_3$  (AP3),  $\beta_5$  chain of integrins (or with control monoclonal IgG1 or polyclonal rabbit IgG; data not shown) and performed as described in the **Methods** section. Biotinylated molecular mass markers (\*) are analyzed on the two external lanes of the gel. Representative gel of three independent experiments.



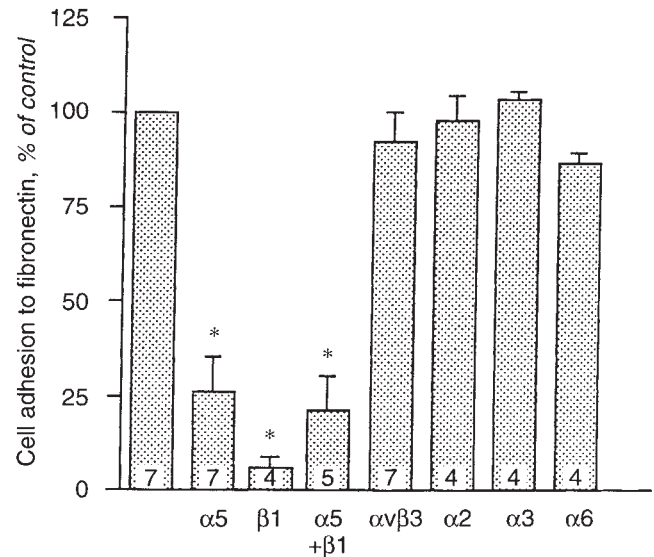


**Fig. 4.** Mesangial cells attachment to various matrix proteins. Mesangial cells were allowed to attach for 60 minutes to 96-well plates, coated with either fibronectin (FN), vitronectin (VN) or vWF, at various concentration (0.1 to 100  $\mu$ g/ml). Adhesion assays were performed as described in the **Methods** section. Results are expressed as the percentage of adherent cells compared with the total number of cells offered. Symbols are: ( $\square$ ) BSA; ( $\diamond$ ) FN; ( $\circ$ ) VN; ( $\triangle$ ) vWF.

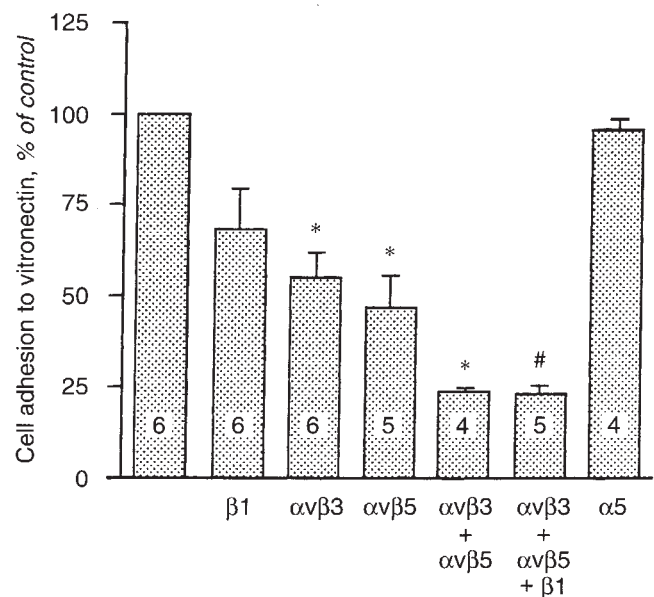
the  $\beta_3$  and  $\alpha_v$  chains and to the  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  complexes. As shown on Figure 1a, HMC are labeled by these four mAbs, thus showing that mesangial cells express both  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrins. In addition,  $\beta_1$  integrins are known to be expressed on mesangial cells [13, 35]. We confirmed these data by showing a positive labeling with antibodies directed against  $\alpha_2$ ,  $\alpha_5$  and  $\alpha_6$  chains (Fig. 1b).

#### Immunoprecipitation and Western blotting

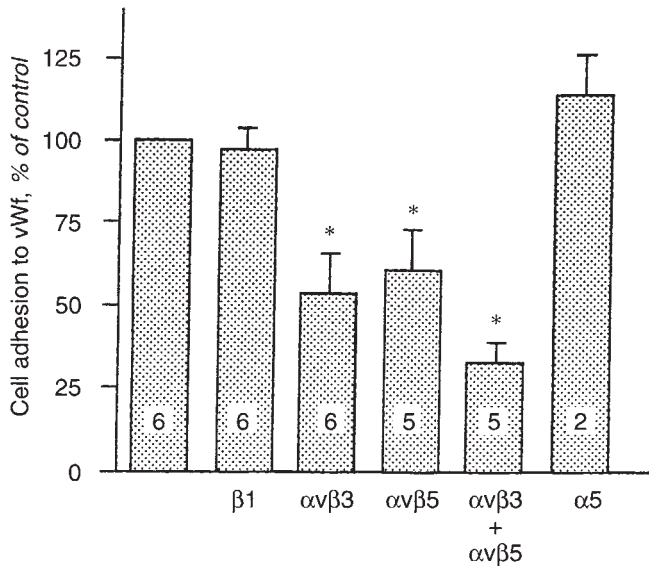
To determine the molecular weight of the  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  complexes identified on the cell surface, mesangial cell membranes were radiolabeled with  $^{125}$ I and immunoprecipitated with specific antibodies to the  $\alpha_v$  and  $\beta_3$  chains and to the  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  complexes. The resulting fractions were subjected to PAGE electrophoresis and autoradiography. Endothelial cells were treated in the same conditions, as a control. As shown in Figure 2A, antibodies to the  $\alpha_v$  and  $\beta_3$  chains or to the  $\alpha_v\beta_3$  complex immunoprecipitated proteins with molecular weight of 97 kDa and 140 kDa, corresponding to the known molecular weights of  $\beta_3$  and  $\alpha_v$  subunits, respectively. Immunoprecipitation of  $\alpha_v$  and  $\beta_3$  chains from mesangial cells produced bands identical to that observed with endothelial cells (Fig. 2B). In addition to  $\alpha_v$  and  $\beta_3$  chains, the anti- $\alpha_v$  antibody precipitated, on mesangial cells (as well as on endothelial cells), a third band with an apparent molecular weight of 115 kDa, which may correspond to the  $\beta_1$  chain. The antibody to  $\alpha_v\beta_5$  complex, which binds to mesangial cells as assessed by FACS analysis, failed to immunoprecipitate this integrin (Fig. 2A).  $\beta_5$  chain expression was nevertheless



**Fig. 5.** Specific inhibition of mesangial cell attachment to fibronectin by anti-integrin  $\alpha_5$  and  $\beta_1$  antibodies. Ninety-six-well plates were coated with fibronectin at 1  $\mu$ g/ml and unbound sites were blocked with BSA. Cells were preincubated for 30 minutes with anti  $\alpha_5$  (clone 16), anti- $\alpha_v\beta_3$  (LM609), anti- $\alpha_2$  (Gi9), anti- $\alpha_3$  (P1B5) and anti- $\alpha_5$  + anti- $\beta_1$  (Lia 1/2). Adhesion assay was then performed as described in the **Methods** section. Results are expressed as percentage of adherent cells compared with the control in presence of a murine monoclonal IgG1. Each point represents the mean  $\pm$  SEM of the number of experiments (N is indicated for each antibody tested). \* $P < 0.05$  versus control.



**Fig. 6.** Specific inhibition of mesangial cell attachment to vitronectin by anti-integrin  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ . Vitronectin at 0.5  $\mu$ g/ml was placed into 96-well plates and non specific sites were blocked with BSA. Cells were preincubated for 30 minutes with anti- $\alpha_v\beta_3$  (LM609), anti- $\alpha_v\beta_5$  (P1F6), anti- $\alpha_5$  (16), anti- $\beta_1$  (Li1/2). Adhesion assay was performed as described in **Methods**. Results are expressed as percentage of adherent cells compared with the control in presence of murine monoclonal IgG1. Each point represents the mean  $\pm$  SEM of the number of experiments (N is indicated for each antibody tested). \* $P < 0.05$  versus control, # $P < 0.05$  versus control and versus inhibition by anti- $\alpha_v\beta_3$  alone.



**Fig. 7.** Specific inhibition of mesangial cell attachment to von willebrand factor by anti-integrin  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ . Von Willebrand factor at 5  $\mu$ g/ml was placed into 96-well plates and nonspecific sites were blocked with BSA. Cells were preincubated for 30 minutes with anti- $\alpha_v\beta_3$  (LM609), anti- $\alpha_v\beta_5$  (PIF6),  $\alpha_5$  (16),  $\beta_1$  (Li1/2). The adhesion assay was performed as described in the **Methods** section. Results are expressed as percentage of adherent cells compared with the control in presence of murine monoclonal IgG1. Each point represents the mean  $\pm$  SEM of the number of experiments (*N* is indicated for each antibody tested). \**P* < 0.05 versus control.

demonstrated by Western blot revealed with an polyclonal antibody to the  $\beta_5$  chain, as shown on Figure 3. The molecular weight of the  $\beta_5$  chain was analogous to that of  $\beta_3$  chain, as already known [10]. In this Western blot analysis (Fig. 3), HUVEC and cultured human fibroblasts, used as control cells, expressed respectively  $\alpha_v\beta_3$  and both  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ , as already known [2].

#### Role of integrins in HMC adhesion to fibronectin, vitronectin and vWf

Mesangial cells were seeded, in the absence of serum, on fibronectin, vitronectin or vWf coated 96-wells plates. Cell adhesion occurred in a dose dependent manner. As shown in Figure 4, 60% of the maximal response was observed at concentrations of about 1  $\mu$ g/ml for fibronectin, 0.5  $\mu$ g/ml for vitronectin and 5  $\mu$ g/ml for vWf. Maximal adhesion was 100% of the offered cells on fibronectin or vitronectin and 80% on vWf. Adhesion experiments performed in the presence of 10% FBS led to similar results to those obtained in the absence of serum. In order to determine which integrins were involved in adhesion to these ligands, we performed inhibition experiments with blocking anti-integrin antibodies. In the following experiments, ascites fluid containing mAbs or purified mAbs were used at a concentration equal or up to five times the concentration, giving the maximal signal in flow cytometry and equal or up to two times the concentration giving the maximal adhesion inhibition in preliminary experiments.

**Adhesion to fibronectin.** As shown in Figure 5, mesangial cell adhesion to fibronectin was strongly inhibited ( $\geq 75\%$  inhibition) by pre-incubation with mAb to the  $\alpha_5$  or to the  $\beta_1$  chain alone and with a mixture of both mAbs. In contrast, neither mAb to  $\alpha_v\beta_3$

complex and  $\alpha_v\beta_5$  complex (data not shown) or to  $\alpha_2$ ,  $\alpha_3$  and  $\alpha_6$  chains had any effect on cell adhesion to fibronectin. These results demonstrate that mesangial cell adhesion to fibronectin was mainly mediated by the  $\alpha_5\beta_1$  integrin.

**Adhesion to vitronectin.** As shown in Figure 6, mesangial cell adhesion was inhibited by pre-incubation with mAb to  $\alpha_v\beta_3$  or to the  $\alpha_v\beta_5$  complexes. When used in combination, these two antibody showed a significant additive effect and strongly inhibited cell adhesion by about 75%. In contrast, mAbs to  $\alpha_5$  had no effect on adhesion to vitronectin. Anti- $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_6$  chains also had no effect (data not shown). The slight inhibition of cell adhesion by anti- $\beta_1$  mAb was not statistically significant and was not additive to that observed with anti- $\alpha_v\beta_3$  and anti- $\alpha_v\beta_5$ . These results demonstrate that adhesion of HMC to vitronectin was primarily mediated by  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrins.

**Adhesion to von Willebrand factor.** Figure 7 shows that mesangial cell adhesion to von Willebrand factor was reduced by about 46% by pre-incubation with antibody to the  $\alpha_v\beta_3$ . Antibody to  $\alpha_v\beta_5$  complexes resulted in about 40% inhibition of cell attachment. These results demonstrate that mesangial cell adhesion to von Willebrand factor involves  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$ .

#### Discussion

We have demonstrated by flow cytometry and immunoprecipitation that  $\alpha_v\beta_3$ , a broadly distributed integrin (particularly on endothelium, smooth muscle cells and fibroblasts [2]), is expressed on HMC and plays a role in mesangial cell adhesion to vitronectin and vWf. The absence of  $\alpha_v\beta_3$ -mediated binding to fibronectin, observed in this study with HMC, is analogous to that observed with human umbilical vein endothelial cells [25], but differs from human dermal microvascular endothelial cells, which bind to fibronectin through  $\alpha_v\beta_3$  [36]. One may hypothesize that the interaction between fibronectin and  $\alpha_v\beta_3$  is not detected on HMC, because its strength is negligible as compared with that of  $\alpha_5\beta_1$ .

$\alpha_v\beta_5$  is also present on HMC, as shown by flow cytometry.  $\alpha_v\beta_5$  was not detected after immunoprecipitation, probably because of the lack of sensitivity of this method and the low amount of  $\alpha_v\beta_5$  present on HMC in culture. Conversely, its presence was directly demonstrated by Western blotting after amplification by chemiluminescence.  $\alpha_v\beta_5$  on HMC binds mainly to vitronectin, as already shown for other cell types such as fibroblasts and carcinoma cells [2, 10]. We observed that  $\alpha_v\beta_5$  on HMC interacts with vWf, since addition of a monoclonal antibody to the  $\alpha_v\beta_5$  complex is inhibitory and increases the inhibition of cell attachment observed with a monoclonal antibody to the  $\alpha_v\beta_3$  complex. This differs from previous reports, which demonstrated that  $\alpha_v\beta_5$  purified from placenta binds to vitronectin, marginally to fibronectin but not to vWf [10], and that a lung adenocarcinoma cell line expressing  $\alpha_v\beta_5$  but not  $\alpha_v\beta_3$  binds to vitronectin but not to fibronectin or to vWf. This apparent discordance with our results on HMC may be due to the fact that the  $\alpha_v\beta_5$ -vWf interaction is weak and not sufficient to promote adhesion to vWf by itself in these models. Our results suggest that  $\alpha_v\beta_5$  on HMC interacts with vWf and could play a role by increasing the strength of  $\alpha_v\beta_3$ -mediated cell attachment to vWf.

Finally,  $\alpha_v\beta_1$  is likely to be present since we observed that the  $\beta_1$  chain coprecipitated with the  $\alpha_v$  chain.  $\alpha_v\beta_1$  has a more restricted distribution, limited to few cell types including fibroblasts, than  $\alpha_v\beta_3$ , and it binds to vitronectin and fibronectin on those cells [2, 9, 37]. By contrast with what was observed on fibroblasts, we

showed that  $\alpha_v\beta_1$  present on human cultured mesangial cells is probably marginally important for fibronectin binding, since a monoclonal antibody to the  $\alpha_5$  chain almost completely inhibited the binding to fibronectin, as already shown by others [13]. Furthermore,  $\alpha_v\beta_1$  did not play an important role in adhesion to vitronectin since the antibody to the  $\beta_1$  chain had a limited (and not significant) effect on cell attachment to vitronectin. Thus, this slight and not significant inhibition observed with the anti- $\beta_1$  mAb precluded any firm conclusion on the eventual role of  $\beta_1$  integrins during adhesion to vitronectin.

The presence of integrins of the  $\alpha_v$  family on mesangial cells may have a number of physiopathological consequences. Modifications of this integrin expression have been reported in glomerular diseases [38]. During mesangial proliferation in IgA nephropathy, the  $\alpha_v\beta_3$  mesangial expression was shown to be increased when using polyclonal Abs [18, 39, 40], or to be decreased when using mAbs (N. Patey, personal communication). In human crescentic GN, we and others have observed that among other integrins,  $\alpha_v\beta_3$  was strongly expressed by the epithelial crescentic cells [17, 41]. Mesangial cells are in close contact with the basal site of endothelial cells [42]. vWf is expressed by all renal tissue endothelial cells, is up-regulated during early fibrosis, and colocalized with TSP in vasculitis, diabetic nephropathy, focal sclerosis and membranous GN, but not in minimal change or IgA nephropathies [16]. On the other hand, other ligands of the  $\alpha_v$  integrin family are also present within the glomerulus and are modified during glomerulonephritis. Indeed, thrombospondin [16, 43, 44], and osteopontin [24, 45] are synthesized by mesangial cells, and vitronectin colocalizes with the complement membrane attack complex in glomerular diseases [46, 47].

One may hypothesize that, *in vivo*, excretion of the vWf at the basal site of endothelial cells [21] and vitronectin deposition [48] would promote, mainly via an interaction with  $\alpha_v\beta_3$ , not only cell adhesion but also migration and cells proliferation. Indeed,  $\alpha_v\beta_3$  has multiple functions possibly related to this potential mechanism and, in addition to adhesion, migration and cell survival,  $\alpha_v\beta_3$  may also play a role in phagocytosis of apoptotic cells in the inflamed glomerulus. This function of  $\alpha_v\beta_3$  has been demonstrated for macrophages but remain to be studied in mesangial cells, which are also able to phagocytize apoptotic neutrophils [49].

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