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Human skin mast cells adhere to vitronectin via the $\alpha v \beta_3$ integrin receptor (CD51/CD61)

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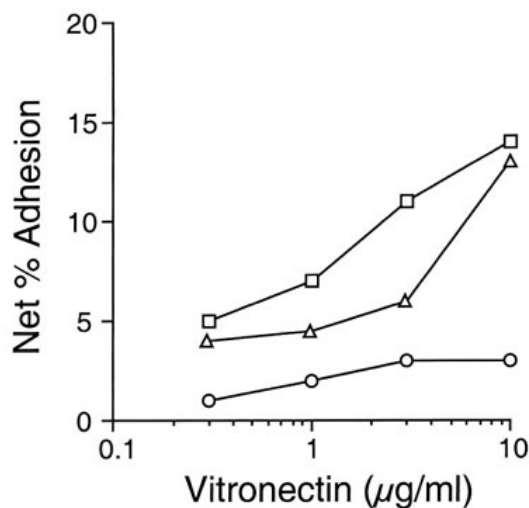
Human mast cells localize within tissues on the basis of their ability to interact with extracellular matrix proteins, such as laminin, fibronectin, and vitronectin. This interaction is mediated by specific cell receptors called *integrins*. For example, human mast cells isolated from adult skin adhere to laminin and fibronectin through the expression of the β_1 integrins VLA-3, VLA-4, and VLA-5.¹ Other studies using murine mast cells, the human mast cell line HMC-1, or mast cells derived from fetal tissues have yielded conflicting results regarding the ability of these cells to adhere to another important extracellular matrix protein, vitronectin, and to express integrins that bind this protein.²⁻⁵ We therefore assessed whether mast cells from human adult skin adhere to vitronectin and identified the receptor involved in this interaction.

Methods for human skin mast cell isolation, identification, and adhesion have been described previously, as have methods for dual-color immunofluorescence and flow cytometry.¹ Vitronectin was obtained from Collaborative Research (Bedford, Mass). The

following integrin mAbs were used: VLA-3 and VLA-5 (CD49c and CD49e; Life Technologies, Grand Island, NY), α_v integrin (CD51, AMF7), β_1 integrin (CD29, 4B4), β_3 integrin (CD61, SZ21), VLA-4 (CD49d; Beckman Coulter, Brea, Calif), and $\alpha_v\beta_3$ (LM 609; Chemicon International, Temecula, Calif); control irrelevant mouse IgG was also used (Becton Dickinson, Sunnyvale, Calif). Results were analyzed by a paired *t* test and are expressed as mean $\pm$ SEM from duplicate determinations.

Fig 1 shows that human adult skin mast cells adhere to vitronectin, with maximal net adhesion at 10 $\mu\text{g/mL}$ ($10\% \pm 3.5\%$).

Fig. 1. Adhesion of human skin mast cells to plastic wells coated with increasing concentrations of vitronectin (0.3-10 $\mu\text{g/mL}$). Cells were incubated for 60 minutes at 37°C. Adhesion to wells coated with BSA (control) was $7\% \pm 2\%$ and was subtracted to yield the net percentage of adhesion. The 3 curves represent data obtained from duplicate determinations in individual donors.



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By flow cytometry, human cutaneous mast cells expressed α_v and β_3 receptors (net fluorescence = 18 and 17, respectively, $n = 1$) similar to human lung and uterine mast cells. In the same experiments, CD49c, CD49d, and CD49e integrins, as well as the common β_1 chain, were also expressed (net fluorescence = 84, 5, 5, and 140, respectively). Although expression of α_v and β_3 was also found on HMC-1 cells and in fetal tissue-derived mast cells, pretreatment with an mAb directed to these integrins failed to inhibit adhesion to vitronectin in the latter.⁵ We pretreated human skin mast cells with an mAb to $\alpha_v\beta_3$ (LM 609) for 10 minutes and tested adhesion to vitronectin (5 $\mu\text{g/mL}$) for 60 minutes. LM 609 significantly inhibited human mast cell adhesion to vitronectin (from 7.2 ± 2.2 to 3.5 ± 1.4 net

percent adhesion, $n = 4$, $P < .05$). An mAb recognizing the β_3 chain (SZ21) also inhibited adhesion to vitronectin (from 6.5 ± 4.6 to 3 ± 3 net percent adhesion, $n = 2$).

These data demonstrate that human adult skin mast cells adhere to vitronectin. This confirms results obtained with mast cells derived from human fetal liver and the HMC-1 cell line.^{3 5} Adhesion was mediated in part by the $\alpha v \beta_3$ receptor. In our previous studies, human mast cell adhesion to fibronectin and laminin was partially inhibited by mAb to VLA-3, VLA-4, and VLA-5 integrins.¹ Together, these findings suggest mechanisms by which integrins contribute to human mast cell localization within tissues.

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