

Fibronectin receptor integrins are involved in mast cell activation

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Mast cells express fibronectin-receptor integrins on the cell surface, which are involved in cellular activation. In this study rat and mouse mast cells adhered to fibronectin through very late antigen 4, 5 ($\beta 1$ integrin) and vitronectin receptor ($\beta 3$ integrin), and engagement of these receptors promoted cellular degranulation induced by cross-linking of the high-affinity IgE receptor. Blocking of these adhesion molecules by monoclonal antibodies remarkably reduced passive cutaneous anaphylaxis reaction in vivo. On fibronectin, cytokine release from mast cells on IgE receptor aggregation was also enhanced, but not the expression of cytokine genes, with the exception of interleukin-3. Interleukin-3 gene expression was constitutively observed in mouse-cultured mast cells and significantly increased on fibronectin with a prolonged survival of the cells, suggesting that the autocrine or paracrine system of interleukin-3 secretion contributes to the prolonged survival of mast cells on fibronectin. Our findings presented here clearly indicate that the engagement of fibronectin-receptor integrins on mast cells increases the sensitivity of the cells for cellular activation. Taking into consideration the fact that mast cells in the microenvironment are actually surrounded by other cells and extracellular matrix proteins, we identified significant roles of adhesion molecules on mast cells in the allergic state, and we hope to develop new strategies to manipulate these molecules for medical intervention in allergy. (J ALLERGY CLIN IMMUNOL 1994;94:625-8.)

Key words: Mast cell, adhesion molecule, integrin, fibronectin, laminin, Fc ϵ RI, cytokine, degranulation, allergy, mast cell survival

Adhesion molecules of immunoglobulin and integrin superfamilies are implicated not only in leukocyte interaction and migration but also in cell activation, as demonstrated with T cells.¹ Engagement of the high-affinity IgE receptor (Fc ϵ RI) triggers mast cell activation and this signal transduction via Fc ϵ RI is very similar to that via T-cell receptor/CD3 in T cells, both of which involve protein tyrosine kinase activation, tyrosine phosphorylation of signal-transducing subunits, phospholipase C activation, and subsequent Ca²⁺ mobilization and protein kinase C activation.²⁻⁴ Of the signal-transducing subunits of these receptors, CD3 ζ and Fc ϵ RI γ chains are structurally similar molecules belonging to the same family and have a common motif, containing two tyrosine residues for signal transduction.⁵⁻⁷ It

Abbreviations used

CMCs:	Cultured mast cells
ECM:	Extracellular matrix
Fc ϵ RI:	High affinity IgE receptor
FNR:	Fibronectin receptor
IL:	Interleukin
mAb:	Monoclonal antibody
TNF- α :	Tumor necrosis factor- α
VLA:	Very late antigen
VNR:	Vitronectin receptor

is suggested that tyrosine residues in this motif are phosphorylated by Rous sarcoma oncogene family kinases associating with CD3 ζ or Fc ϵ RI γ chain on receptor engagement.^{8, 9} In addition, ZAP70 and Syk kinase associate with CD3 ζ and the Fc ϵ RI γ chain, respectively, on receptor cross-linking.^{10, 11} These common events, observed in an early signal transduction pathway of T-cell receptor/CD3 and Fc ϵ RI, provide a good rationale for testing the hypothesis that signals through adhesion molecules on mast cells also modulate mast cell activation, as in T cells.

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0091-6749/94 \$3.00 + 0 1/0/57119

Mast cells in peripheral tissues are actually surrounded by other cells, such as fibroblasts and mucosal cells and extracellular matrix (ECM) proteins (e.g., collagen, fibronectin, laminin), suggesting that mast cells adhere to these cells and ECM, receiving some signals through adhesion molecules expressed on the cell surface.¹² Therefore to investigate the process of allergic inflammation *in vivo*, we cannot neglect the effect of integrin-mediated signals on mast cell activation.

ADHESION OF MAST CELLS TO ECM PROTEINS

We first examined the adherence of mast cells to various ECM proteins coated on a plate. A rat mucosal mast cell line, RBL-2H3, bound to fibronectin, vitronectin, and fibrinogen without any stimuli, but not to laminin or collagen. Freshly prepared rat peritoneal mast cells exhibited a similar binding specificity to ECM proteins but only after phorbol myristate acetate (PMA) stimulation as in the case of resting T cells in which the $\beta 1$ and $\beta 2$ integrin-mediated adhesion was activated by PMA.¹³⁻¹⁵ A mouse connective tissue type mast cell line, PT18, bound only to fibronectin and not to vitronectin, fibrinogen, laminin, or collagen. Mouse bone marrow-derived mast cells cultured with interleukin (IL)-3 bound to fibronectin, vitronectin, and to a lesser extent, to laminin when stimulated with PMA.

The fibronectin adherence of RBL-2H3 and RPMCs was inhibited by both the connecting segment (CS)-1 peptide of fibronectin that blocks very late antigen (VLA)-4 interaction with fibronectin and the RGD peptide of fibronectin that blocks VLA-5 and vitronectin receptor (VNR) interaction with fibronectin. In the case of PT18, fibronectin adherence was strongly inhibited by the RGD peptide and weakly inhibited by the CS-1 peptide.

EXPRESSION OF INTEGRINS ON MAST CELLS

We confirmed the expression of receptors on mast cells for ECM proteins by fluorescence-activated cell sorter analysis with anti-integrin monoclonal antibodies (mAbs) generated in our laboratory. RBL-2H3 expressed VLA- $\alpha 4$, VLA- $\alpha 5$, and VNR($\beta 3$) on the cell surface, and rat peritoneal mast cells also expressed VLA- $\alpha 4$, VNR($\beta 3$), and to a lesser extent, VLA- $\alpha 5$. Anti-VLA- $\alpha 4$, anti-VLA- $\alpha 5$, and anti-VNR($\beta 3$) mAbs inhibited fibronectin adherence of both RBL-2H3 and rat peritoneal mast cells, and the strongest

inhibitory effect was observed with the mixture of all these antibodies, which was consistent with the above mentioned inhibitory effects of RGD and CS-1 peptides.

Both PT-18 and cultured mast cells (CMCs), primarily cultured mouse mast cells, expressed VLA- $\alpha 4$, VLA- $\alpha 5$, and VNR($\beta 3$) on their cell surface like rat mast cells. On CMCs, the expression of VLA- $\alpha 1$, VLA- $\alpha 2$, and VLA- $\alpha 6$ as laminin receptors was also observed. Fibronectin adherence of PT18 was strongly inhibited by anti-VLA- $\alpha 5$ and anti-VNR($\beta 3$) mAbs as by the RGD peptide, and weakly inhibited by anti-VLA- $\alpha 4$ mAb as by the CS-1 peptide, indicating that VLA-5 is dominant as a fibronectin receptor (FNR) in PT18. With the mixture of these mAbs, the strongest inhibition was observed in PT18. Fibronectin adherence of CMCs was weakly blocked by each antibody [anti-VLA- $\alpha 4$, VLA- $\alpha 5$, VNR($\beta 3$)] and strongly blocked by the mixture of these antibodies. Laminin adherence of CMCs was weakly blocked by anti-VLA- $\alpha 2$, anti-VLA- $\alpha 6$ mAbs and strongly blocked by the mixture of these mAbs.

These results indicate that the adherence of mast cells to ECM proteins is mediated through the specific interaction of integrin molecules with ECM proteins.

ENHANCEMENT OF MAST CELL DEGRANULATION BY THE ENGAGEMENT OF FIBRONECTIN RECEPTOR INTEGRINS

Exocytosis of β -hexosaminidase as an indicator of degranulation from RBL-2H3 in response to Fc ϵ RI engagement with IgE and anti-IgE antibody was greatly enhanced on fibronectin-coated plates specifically but not on collagen- and bovine serum albumin-coated plates. This enhancement of exocytosis on fibronectin was blocked by RGD and CS-1 peptides or by anti-VLA- $\alpha 4$, anti-VLA- $\alpha 5$, and VNR mAbs. Fab fragments of these antibodies were as effective as whole antibodies, indicating that this inhibition was caused by the real blocking of the interaction between fibronectin and integrins and not by some modulatory signals through Fc γ receptors. Taken together, these findings confirmed that FNR-mediated specific interaction with fibronectin promotes mast cell activation by Fc ϵ RI engagement.

The involvement of FNR in mast cell activation was also investigated *in vivo*. Local administration of the mixture of anti-VLA- $\alpha 4$, anti-VLA- $\alpha 5$, and anti-VNR($\beta 3$) mAbs greatly reduced the passive cutaneous anaphylaxis reaction in rats, indicating

that these antibodies dissociate mast cells from ECM or some other cells, such as fibroblasts in a local microenvironment. Enhancing signals for mast cell degranulation through integrins on the cell surface may play a significant role in allergic state, especially when the IgE or antigen level is low.

INVOLVEMENT OF FNR INTEGRINS IN CYTOKINE PRODUCTION OF MAST CELLS

It is now well established that mast cells are also important as a source of various cytokines, such as IL-3, IL-4, IL-5, tumor necrosis factor (TNF)- α , granulocyte-macrophage colony-stimulating factor, and interferon- γ , inducing allergic inflammation.¹⁶

Among these cytokines produced by mast cells, TNF- α and IL-4 are of great importance because they induce adhesion molecules on vascular endothelial cells in inflamed lesions to initiate the migration of inflammatory cells such as granulocytes and lymphocytes.

We first assessed the involvement of FNR integrins in TNF- α production and release by PT18 cells, which contain a large amount of TNF- α . PT18 cells on fibronectin with Fc ϵ RI engagement released TNF- α approximately 2.5 times more than PT18 cells on collagen, although almost no release of TNF- α was observed without Fc ϵ RI engagement. RGD peptide abolished this enhancement of TNF- α release on fibronectin, and CS-1 peptide inhibited this enhancing effect to a lesser extent, indicating that VLA-5 is dominant in enhancement of TNF- α release from PT18 cells on fibronectin.

We next examined TNF- α gene expression in PT18 cells by the reverse transcriptase-polymerase chain reaction method. The TNF- α gene was constitutively expressed without any stimuli. Cross-linking of Fc ϵ RI by IgE-anti-IgE antibody complexes greatly promoted TNF- α gene expression of PT18 cells to the same extent, whether the cells were on fibronectin or collagen. Together with the results mentioned above, it was indicated that signals through FNR integrins on PT18 cells enhance TNF- α release but have no effect on TNF- α gene expression.

Cytokine gene expression in CMCs was also investigated by the reverse transcriptase-polymerase chain reaction method. IL-3, IL-4, and granulocyte-macrophage colony-stimulating factor genes were constitutively expressed without any stimuli. With Fc ϵ RI engagement, the expression of IL-3 and IL-4 genes was greatly enhanced, but

granulocyte-macrophage colony-stimulating factor gene expression was not enhanced at all. Interestingly, only IL-3 gene expression was enhanced in CMCs on fibronectin, suggesting that CMC survival should be prolonged by the autocrine/paracrine system of IL-3 when the cells are on fibronectin.

PROLONGED SURVIVAL OF MAST CELLS ON FIBRONECTIN

CMCs were derived from mouse bone marrow and cultured with IL-3 for 4 weeks, and then the CMC survival rate on fibronectin was estimated. Survival rate of CMCs on fibronectin was significantly higher than that on collagen and bovine serum albumin, whether Fc ϵ RI were engaged or not. This prolongation of CMC survival on fibronectin was blocked by anti-VLA- α 4, anti-VLA- α 5, and anti-VNR(β 3) mAbs, indicating that this prolonged survival was really specific for FNR integrins' engagement on the cell surface. Taken together, our findings suggest that the IL-3 autocrine/paracrine system of mast cells on fibronectin in peripheral tissues may play an important role in mast cell survival.

CONCLUDING REMARKS

We documented here the involvement of FNR integrins in mast cell activation. Although without cross-linking of Fc ϵ RI, mast cells on ECM proteins were not activated to degranulation, our findings suggest that FNR integrins' engagement on the cell surface enhances the sensitivity of mast cell activation and plays a significant role, especially when antigen and IgE stay at low levels in a microenvironment. Another interesting finding is that the prolonged survival of mouse mast cells on fibronectin is probably due to the IL-3 autocrine/paracrine system. Elucidation of the molecular mechanisms that underlie the phenomena we have found will enable us to further explore the allergic state and give us fine tools for the medical manipulation of allergy.

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