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Characterization of adhesive interactions between mast cells and laminin isoforms: evidence of a principal role for α6 integrin

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SUMMARY

Mast cells are known to adhere to laminin, although there is limited information on the characteristics of this event. To further examine this adhesive interaction, we thus determined the adherence of murine mast cell lines and primary bone marrow cultured mast cells (BMCMC) to murine laminin (mLN), human placental laminin-1 (hLN), merosin (laminin-2) and various laminin fragments, concentrating on activating stimuli, the involvement of integrins, and the effect on mast cell activation. Murine mast cells were found to adhere to both mLN and hLN and to merosin, not only following exposure to phorbol 12-myristate 13-acetate (PMA), but also after FcεRI aggregation or addition of stem cell factor (SCF). Adhesion to laminin was partially inhibited by soluble E8 and PA22-2, both fragments of laminin that promote mast-cell adhesion when bound on surfaces. Mast-cell lines and BMCMC consistently expressed high levels of α6 integrin. Antibody to α6 blocked spontaneous and inhibited activated mast-cell adhesion to hLN, and inhibited mast-cell adhesion to mLN and its fragment E8. Mast-cell adhesion to both laminin isoforms increased FcεRI-mediated mast-cell secretion. These observations demonstrate that mast-cell attachment to laminin is promoted by physiological stimuli, is mediated principally by α6 integrin, and results in enhanced cell activation.

INTRODUCTION

Mast cells reside in connective tissues and increase at sites of tissue injury and inflammation, where these cells presumably play a regulatory role both in inflammatory responses and tissue repair. In non-pathological conditions, mast cells occur in association with small vessels, nerves and glands, a distribution attributed in part to the presence of laminin associated with these structures.[1] In fact, the earliest reports that mast cells could adhere to connective tissue components employed laminin in the adhesion assays.[2–3] While mast cells are now known to adhere to other matrix components including fibronectin[4] and vitronectin,[5] interactions between mast cells and laminin could be particularly relevant to understanding the biology of mast cells.

In other systems, laminin has been shown to mediate various biological activities including the attachment and spreading of eosinophils,[6] natural killer (NK) cells,[7] neutrophils,[8] monocytes[9] and T and B lymphocytes.[10] Laminin induces differentiation of human endothelial cells into capillary-like structures,[11] stimulates neurite outgrowth[12] and induces proliferation of thymocytes stimulated through CD3.[13] The ability of laminin to alter the biology of multiple cell types seems in part to result from the complexity of the laminin molecule and in part, to depend upon the potential of various cells to interact with laminin through an array of surface receptors. These receptors include members of the β1 integrin family with various α subunits, the principal of which seems to be α6.

There is, however, relatively little known about how mast cells interact with laminin, and about the biological effects of this interaction, with the exception that an antiserum to a 67 000 MW surface protein, identified on murine melanoma cells as a laminin receptor,[14] partially inhibits adhesion of mast cells to murine laminin (mLN).[3] Thus, to further characterize the interactions between mast cells and laminin we examined the ability of mast cells to adhere to laminin isoforms and laminin fragments, and explored the role of integrins in these interactions. We also examined the potential of mast cell–laminin interactions to alter the biological responsiveness of mast cells as determined by changes in granule exocytosis after adhesion.

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Abbreviations: BMCMC, bone marrow cultured mast cells; hLN, human placental laminin; mLN, murine laminin; SCF, stem cell factor.

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MATERIALS AND METHODS

Materials

Mouse laminin from basement membrane from Engelbreth–Holm swarm mouse sarcoma (mLN) and WEHI-3 (mouse

myelomonocytic cell line) conditioned medium [containing interleukin-3 (IL-3)] (Collaborative Biomedical Products, Bedford, MA); DMEM, RPMI-1640, penicillin/streptomycin, HEPES, L-glutamine, non-essential amino acids (Biofluids Inc., Rockville, MD); 96-well Immulon 1 plates (Dynatech Laboratories Inc., Chantilly, VA); peptide PA22-2 (Bachem, Torrance, CA); *N*-hydroxysulphosuccinimide ester of biotin (NHS-LC–biotin), streptavidin–horseradish peroxidase (HRP) (Pierce, Rockford, IL); human placental laminin (hLN), merosin, recombinant protein-G agarose, Trizol, Superscript Reverse Transcription (RT) kit, Taq polymerase (Life Technologies, Gaithersburg, MD); murine recombinant stem cell factor (mrSCF) (R&D Systems, Minneapolis, MN); monoclonal rat anti-α6 antibody, clone GoH3 (Immunotech Inc., Westbrook, ME); immunoglobulin E (IgE) monoclonal anti-dinitrophenyl (DNP) antibody (clone SPE-7), phorbol 12-myristate 13-acetate (PMA), DNP–human serum albumin (HSA), 2-mercaptoethanol (2-ME) (Sigma Chemical Company, St. Louis, MO); Econofluor scintillation fluid, [methyl-^{3}H]thymidine (6·7 Ci/mmol) (NEN Du Pont Company, Boston, MA); tris–glycine 6% gels (Novex Technologies, San Diego, CA); and anti-mouse β1 antibody, clone 9EG7 (Pharmingen, San Diego, CA); were purchased from the manufacturers.

The E8 proteolytic fragment of laminin was a gift from Dr P. Yurchenco (Department of Pathology, Robert Wood Johnson Medical School, Piscataway, NJ, USA) and the anti-α1 and anti-α2 integrin (hamster anti-mouse) antibodies a gift from Dr H. Yagita (Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan).

Cell cultures

C57 mast cells are IL-3 independent, and were derived from the bone marrow of BALB/c mice.[15] These cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4 mM L-glutamine, 100 μg/ml penicillin/streptomycin, 50 μM 2-ME and 10% heat inactivated fetal calf serum (FCS). Bone marrow cultured mast cells (BMCMC) were obtained as described[16] from BALB/c mice and cultured in RPMI-1640 medium supplemented with 10% WEHI-3, 1 mM sodium pyruvate, 0·1 mM non-essential amino acids, 4 mM L-glutamine, 25 mM HEPES, 100 μg/ml penicillin/streptomycin, 50 μM 2-ME and 10% FCS. BMCMC were used after 3–5 weeks in culture when greater than 95% of the cells were mast cells as determined by toluidine blue staining. CFTL-15 mast cells are IL-3 dependent and were cultured from the fetal liver of normal NFS/N mice. These cells were cultured in medium used for BMCMC except that it was supplemented with 20% WEHI-3.

FcεRI aggregation

C57 or BMCMC were suspended at 10^6 cells/ml and incubated for 2 hr at 37° with 1 μg/ml of mouse IgE anti-DNP. Subsequently the cells were washed twice with fresh medium, suspended at $2\times10^5-4\times10^5$ cells/ml and FcεRI aggregated by the addition of 20 ng/ml of DNP–HSA. Granule exocytosis was measured by quantification of β-hexosaminidase.[17]

Adhesion assay

Five $\times 10^5$ mast cells/ml in fresh medium containing 10 μCi/ml of [^{3}H]thymidine were cultured for 18 hr in a CO_2 incubator, washed in DMEM supplemented with 1% BSA and resuspended at 4×10^5 cells/ml in the same medium for use in adhesion experiments.

Flat-bottom 96-well plates (Immulon 1) were coated overnight at 4° with 100 μl of a specific protein or peptide diluted in DMEM at the indicated concentrations. This solution was then discarded and non-specific binding blocked with 100 μl of 5% BSA in phosphate-buffered saline (PBS) for 2 hr at 37°. The wells were rinsed twice with PBS and used in adhesion experiments. Preliminary experiments verified that maximum adhesion of mast cells to laminin was obtained at a laminin coating concentration of 10 μg/ml as reported.[2]

Four $\times 10^5$ cells in 100 μl of DMEM with 1% BSA were added in each well alone for spontaneous adhesion; or together with activating agents for given time intervals. The non-adherent cells and medium were then aspirated, the wells washed twice with 150 μl of DMEM with vigorous shaking and the non-adherent cells aspirated from each well during washing were pooled in new wells. The adherent cells, as well as the pooled non-adherent cells were harvested with an automatic cell harvester (Cambridge Technologies Inc., Watertown, MA). Incorporated radioactivity in the adherent and non-adherent fractions was measured with a 2000CA liquid scintillation analyser (Packard Instrument Company, Downers Grove, IL). The percentage adhesion was calculated according to the following formula:

$$\%\ \text{cell adhesion} = \frac{\text{radioactivity in adherent cells}}{\text{radioactivity in adherent} + \text{non-adherent cells}} \times 100$$

For inhibition experiments, cells were preincubated for 30 min at 37° with the indicated concentrations of peptides or antibodies and then employed in the adhesion experiments as described.

All assays were performed in duplicate. One value, the mean of the two measurements, was calculated for each condition in each independent experiment. The results in the text are given as the mean ± SEM of n independent experiments.

Flow cytometry

One $\times 10^6$ mast cells were washed twice with PBS with 0·05% NaN_3 and 0·1% BSA and incubated for 30 min with the appropriate primary antibody at 10 μg/ml or an isotype matched control (rat IgG2a for anti-α6 antibody and hamster IgG for anti-α1 and anti-α2 antibodies) in a total volume of 50 μl. The cells were next washed twice with buffer and incubated for 30 min with the appropriate secondary fluorescein isothiocyanate (FITC)-labelled antibody. The cells were then washed twice, resuspended in 500 μl of buffer and analysed by fluorescence-activated cell sorting (FACScan, Becton-Dickinson, Mountain View, CA). All procedures were at 4°.

Biotin labelling of surface proteins

Cells were washed twice with PBS with 1 mM Ca^{2+} and Mg^{2+}, resuspended in the same buffer at 5×10^6 cells/ml and incubated twice for 1 hr with 0·1 mg/ml of NHS-LC–biotin at 4°. After the second incubation, cells were washed four times with PBS with 1 mM Ca^{2+} and Mg^{2+} and 50 mM NH_4Cl to remove unincorporated biotin.

Immunoprecipitation of the α6 and β1 integrin

Surface-biotinylated cells were solubilized with lysis buffer (borate-buffered saline with 1% Triton-X- 100 plus 1 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml aprotinin, 4 μg/ml leupeptin and 10 μg/ml pepstatin). The cell lysate was precleared by incubation with 50 μl of recombinant protein-G agarose at 4° for 3 hr. α6 was immunoprecipitated overnight with 50 μl of recombinant protein-G agarose coupled to 5 μg of anti-α6 antibody (GoH3). Immunoprecipitated proteins from the same number of cells were separated on a 6% polyacrylamide gel under non-reducing conditions[18] and subsequently transferred to a nitrocellulose membrane. Non-specific binding was blocked with 5% BSA in tris-buffered saline. The membrane was then incubated with streptavidin–HRP 3 μg/ml for 1 hr at room temperature and the proteins visualized with enhanced chemiluminescence (ECL) on Kodak film.

Reverse transcription–polymerase chain reaction (RT–PCR) for integrin message

RNA was isolated from mast cells with Trizol according to the instructions and was used for reverse transcription to cDNA with the Superscript RT kit. Two microlitres of the 20 μl RT reaction were used for PCR amplification with Taq polymerase of α3 (40 cycles of 94° for 1 min, 60° for 1 min and 72° for 1 min) and α6 (30 cycles of 94° for 1 min, 55° for 1 min and 72° for 1 min) message. The primers used were: for the amplification of α3 message 5′-TGGAGTGCCC-CCTTCCAGAC-3′ for the sense strand and 5′-GTA-GCGTGCTCCCTGGAGGT-3′ for the antisense strand; and for the amplification of α6 message: 5′-GACTCTT-AACTGTAGCGTGA-3′ for the sense strand and 5′-ATCTCTCGCTCTTCTTTCCG-3′ for the antisense strand. The α6 primers have been shown to give a 550 bp band (α6 A) and a 420 bp band (α6 B).[19] The α3 primers are expected to give a 554 bp band (α3 A) and a 410 bp band (αi3 B). The PCR products were separated on a 1% agarose–Tris–acetate–ethylenediaminetetra-acetic acid buffer (TAE) gel and photographed.

RESULTS

Adhesion of mast cells to mouse and human laminin

It has been reported that mast cells adhere to laminin after activation with PMA. To extend these observations, we studied the adhesion of C57 mast cells and of BMCMC to mLN following activation with physiological stimuli known to activate mast cells to adhere to other substrates.[4–16] As can be seen in Fig. 1, neither C57 (Fig. 1a) nor BMCMC (Fig. 1b) show significant spontaneous adhesion to laminin. As reported, PMA induced ≈45% of either population of mast cells to adhere to laminin. Further, FcεRI aggregation (Fig. 1a and b) was also followed by mast-cell adhesion to laminin. Spreading after adhesion was observed in both mast cell populations. SCF induced BMCMC, but not C57 mast cells, to adhere to laminin. Mast-cell adhesion following PMA stimulation (Fig. 1c) plateaued at ≈60 min, similar to mast cell adhesion to fibronectin. Aggregation of FcεRI resulted in a similar time course of adhesion for the first hour but the adhesion returned to baseline during the subsequent 2 hr (Fig. 1d). Thus, BMCMC adhere to laminin following FcεRI aggregation and after exposure to SCF.

Specific cell types may differ in adhesion to laminin isoforms. Thus we next examined the adhesion of murine mast cells to hLN and human merosin (laminin-2). C57 mast cells exhibited 22±9% spontaneous adhesion and BMCMC 25±9% spontaneous adhesion to wells coated with hLN (data not shown). Following PMA (50 ng/ml) activation, the adhesion of C57 mast cells increased to 42±4% and that of BMCMC to 72±9% (data not shown). FcεRI crosslinking increased the adhesion of BMCMC to hLN to 45%. Activated adhesion to hLN was followed by spreading not evident during spontaneous adhesion. BMCMC also adhered to merosin up to 37·8±4% after activation with PMA (50 ng/ml) (data not shown). Thus, murine mast cells adhere also to hLN (closely homologous to murine laminin-1) and to merosin.

Peptides that represent sequences within the three chains of laminin are reported to inhibit adhesion of specific cells to laminin. One active site is on the proteolytic fragment E8 and near peptide PA22-2 which inhibits the adhesion of B16-F10 mouse melanoma cells to laminin.[12] Thus, to further characterize sites of adhesion of mast cells to laminin, we examined the ability of E8 and PA22-2 to inhibit mast cell adhesion. E8 at 100 μg/ml inhibited BMCMC adhesion to mlN by 23±3% and PA22-2 at 500 μg/ml inhibited the adhesion by 36±4% (data not shown). We also examined mast-cell adherence to these fragments coated on a surface. As can be seen in Fig. 2, while both BMCMC and C57 mast cells had low spontaneous adhesion to E8, adhesion increased to over 25% after activation. Further, both BMCMC and C57 mast cells spontaneously adhered to PA22-2 with maximum adhesion of 40–43% for both C57 cells and BMCMC. Thus, mast-cell adhesion to laminin and E8 is mediated partially through a site found in PA22-2.

Expression of laminin receptors on mast cells

The integrins α1, α2, α3 and α6 function as laminin receptors in other cell types. To determine which of these integrins are expressed on mast cells, we employed FACS analysis for α1, α2 and α6 and RT–PCR for α3 as antibodies to this integrin subunit were not available. C57, CFTL-15 and BMCMC thus express α6 integrin receptors (Fig. 3a, b, c), and this expression is not increased by activation of cells with 50 ng/ml PMA for up to 18 hr (data not shown). No cells express α1 integrins (Fig. 3d, e, f). BMCMC, but not C57 or CFTL-15 mast cells, express α2 integrins (Fig. 3g, h, i). Thus, only α6 was consistently expressed on all mast cells.

The expression of α3 was examined by RT–PCR. C57 cells and BMCMC but not CFTL-15 cells, expressed message for the A form of α3 integrin (Fig. 4a). All mast-cell populations expressed the α6 A isoform of α6 integrin (Fig. 4b). Actin is amplified from all cells as a control (Fig. 4c).

To confirm the presence of α6 on mast cells, we immunoprecipitated the α6 integrin from biotin surface-labelled C57 and CFTL-15 cells with the monoclonal anti-α6 antibody GoH3. CFTL-15, as expected, had a higher level of expression of α6 (Fig. 4d). In both cases a faint band that was co-immunoprecipitated was shown to co-migrate with the principal band immunoprecipitated with an anti-β1 antibody. No bands corresponding to the integrin α chains are seen in

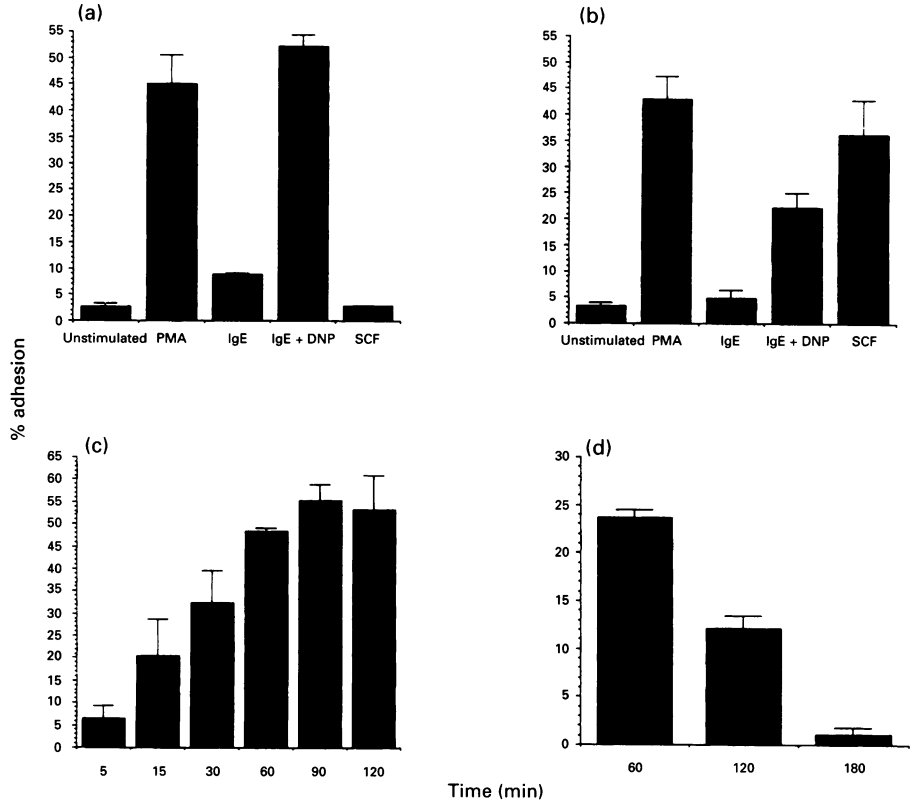

Figure 1. Adhesion of C57 (a) and BMCMC (b, c and d) to mLN. (a) and (b): cells were incubated for 1 hr in wells coated with mlN (10 μg/ml), either resting or after activation with PMA (50 ng/ml), IgE anti-DNP alone or with receptor aggregation with DNP–HSA (10 ng/ml) or SCF (100 ng/ml). (c) Time course of BMCMC adhesion to wells coated with mLN (10 μg/ml) after activation with PMA (50 ng/ml). (d) Time course of BMCMC adhesion to mLN (10 μg/ml) after activation with IgE anti-DNP plus DNP-HSA. Data is presented as mean ± SEM, $n=4$.

immunoprecipitations with anti-β1 antibodies (Fig. 4d lanes 2 and 4). The degree of co-immunoprecipitation of α and β chains in all immunoprecipitations was unimpressive and possibly was caused by a lack of divalent cations in the immunoprecipitating buffer.

Inhibition of mast cell adhesion to laminin and its fragments by anti-α antibodies

To examine the role of α6 integrin in the adhesive interactions between mast cells and laminin, we employed the rat monoclonal anti-α6 antibody GoH3 (Table 1). Preincubation of BMCMC or C57 cells with anti-α6 antibody had a small but significant inhibitory effect on PMA induced adhesion of these cells to mLN (12 ± 2% and 21 ± 7%, respectively) while it inhibited the adhesion of CFTL-15 by 67 ± 15%. Anti-α6 had a greater effect when it was used to inhibit spontaneous adhesion to hLN (91–95%), or PMA-induced adhesion to hLN (52–86%). Anti-α6 also inhibited the adhesion of BMCMC to hLN after FcεRI crosslinking by 38% (data not shown). GoH3 similarly inhibited PMA induced mast-cell adhesion to E8 (35–66%) and almost completely blocked spontaneous mast cell adhesion to PA22-2 (86–87%). Anti-α6 antibody inhibited the spreading of the cells observed during PMA induced adhesion. In all cases, isotype matched control IgG (rat IgG2a), as well as antibodies against the adhesion molecule CD44, and integrin α5 had no effect on the adhesion of mast cells to these proteins. Anti-α6 antibody showed the highest inhibition in the experiments with CFTL-15 mast cells, consistent with the higher expression of α6 on CFTL-15 cells (Fig. 3). Thus, anti-α6 antibody blocked unstimulated adhesion to hLN and PA22-2 and induced significant inhibition of the PMA-induced adhesion.

Mast cell secretion

C57 cells released more β-hexosaminidase following FcεRI aggregation when adherent to plates coated with mLN (25 μg/ml) or hLN (2 μg/ml) compared with plates coated with BSA (Fig. 5a). Soluble PA22-2 (up to 200 μg/ml) as well as the spontaneous adhesion to plate bound PA22-2 (10–25 μg/ml) caused no secretion of β-hexosaminidase from BMCMC; neither had any effect on FcεRI aggregation induced secretion of β-hexosaminidase from C57 or BMCMC (data not shown).

BMCMC also showed increased β-hexosaminidase release following FcεRI aggregation when adherent to plates coated with hLN (2 μg/ml) or merosin (10 μg/ml) compared to plates coated with BSA (Fig. 5b). This increase was evident when suboptimal concentrations of antigen were used for aggregation. Adhesion had no effect on secretion when an optimal concentration of antigen (10 ng/ml) was used to induce secretion from BMCMC (Fig. 5c). Preincubation of mast cells with anti-α6 antibodies inhibited this synergistic effect by 35%.

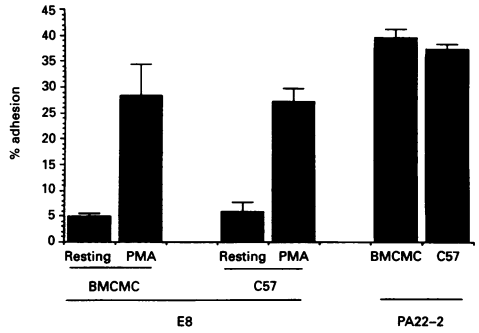

Figure 2. Adhesion of C57 and BMCMC to wells coated with E8 (10 μg/ml) without activation (resting) and after activation with PMA (50 ng/ml) for 1 hr or to PA22-2 (10 μg/ml) without activation. The concentration of E8 and PA22-2 used was shown in preliminary experiments to be associated with maximal adhesion of C57 cells. Data is presented as mean ± SEM, $n = 3$.

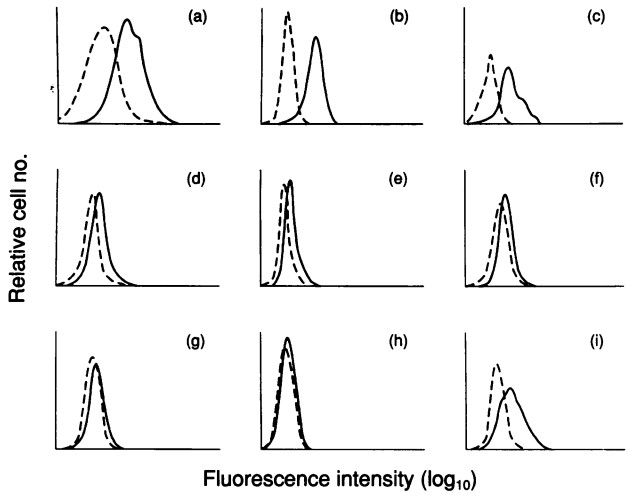

Figure 3. FACS analysis of C57 (a, d, g), CFTL-15 (b, e, h), and BMCMC (c, f, i) for expression of α6 (a, b, c), α1 (d, e, f) and α2 (g, h, i). For details, see materials and methods.

Direct crosslinking of α6 integrins using anti-α6 antibodies plus goat-anti-rat IgG did not increase the FcεRI mediated secretion of β-hexosaminidase from BMCMC, indicating that adhesion was necessary for this effect (data not shown).

DISCUSSION

It is known that the adhesion of mast cells to various connective tissue components varies considerably from mast-cell line to mast-cell line, and that adhesion may be up-regulated following cell activation.[3,16] In the studies reported here, murine mast cells are observed to have low spontaneous adhesion to murine laminin-1 and human laminin-2 and a higher spontaneous adhesion to human laminin-1. Mast-cell adhesion to all forms of laminin increases after activation with PMA and following activation through FcεRI or engagement of c-kit receptor by stem cell factor (Fig. 1). SCF induced adhesion of BMCMC but did not induce adhesion of C57 mast cells to laminin. This observation is consistent with the lack of proliferation of our C57 mast cells in response to SCF (unpublished observation). This could

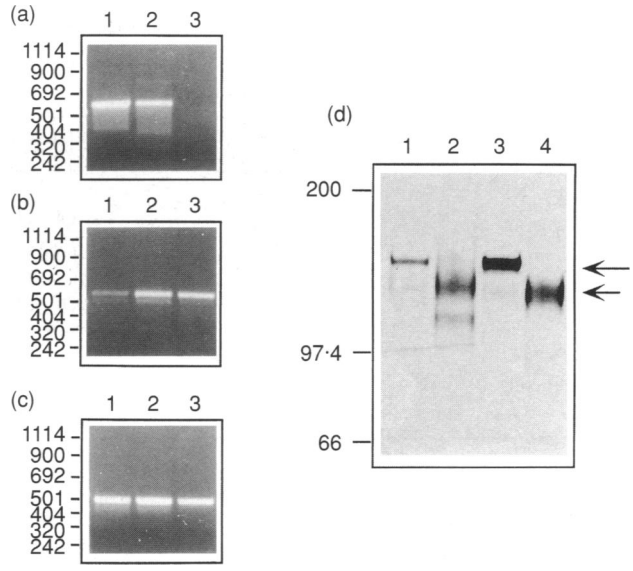

Figure 4. Expression of α3 integrin by murine mast cells. (a) RT–PCR for message of α3 integrin. Lane 1: BMCMC; lane 2: C57; lane 3 CFTL-15. (b) RT–PCR for α6 integrin message. Lane 1: BMCMC; lane 2: C57; lane 3 CFTL-15. (c) RT–PCR for actin. Lane 1: BMCMC; lane 2: C57; lane 3 CFTL-15. (d) Immunoprecipitation of α6 and β1 integrins from surface biotinylated C57 and CFTL-15 mast cells. Cells were biotinylated as described in methods. One × 10^7 cells were then lysed and immunoprecipitated overnight with anti-α6 or anti-β1 antibodies (5 μg). Lysates were run on a 6% polyacrylamide gel, transferred to a nitrocellulose membrane and stained with streptavidin–HRP (3 mg/ml). Lanes 1: C57 cells immunoprecipitated with anti-α6; 2: C57 cells immunoprecipitated with anti-β1; 3: CFTL-15 cells immunoprecipitated with anti-α6; 4: CFTL-15 cells immunoprecipitated with anti-β1. Arrows show location of α6 (long arrow) and β1 (short arrow) bands. Molecular weights are shown on the left vertical axis.

be the result of a non-functional c-kit on C57 mast cells. Thus, the normal adhesion of mast cells in tissues may be dependent on SCF, but mast cells would also be expected to increase adherence to laminin at sites of allergic inflammation. PA22-2 and E8 partially inhibited the adhesion of mast cell to laminin, each no greater than a maximum inhibition of 23–35%. PA22-2 and E8 belong to the long arm of laminin where one of the active sites of laminin has been identified. Thus, it appears that multiple domains of the laminin molecule are required for a complete adhesive interaction between mast cells and laminin.

Activated mouse macrophages adhere to laminin in part through α6β1 integrin (with a 70% inhibition with anti-α6 antibodies),[9] eosinophils appear to adhere to laminin through α6 integrin[6] and human NK cells adhesion to laminin after activation through CD16 crosslinking is inhibited by anti-α6 antibody.[7] The expression of α6 has not been studied in mouse mast cells, although human dermal mast cells[1] but not mast cells cultured from human fetal liver[20] express α6. Data presented in this paper reveal that α6 integrin similarly is a principle receptor for the adhesion of murine mast cells to murine, and in particular, to human laminin-1 (Table 1). Anti-α6 antibody inhibits greater than 90% of spontaneous mast cell adhesion to human laminin-1 and 50–86% of PMA induced adhesion depending upon the mast cell employed in

Table 1. Inhibition of mast cell adhesion to laminin by anti-α6 antibodies (GoH3)*

	Coating substances			
Mast cells	mLN	hLN	E8	PA22-2
BMCMC				
spontaneous	NA	95±1%	NA	87±5%
PMA	12±2%	52±2%	43±11%	NA
C57				
spontaneous	NA	91±2%	NA	86±6%
PMA	21±7%	53±4%	35±11%	NA
CFTL-15				
spontaneous	NA	94±3%	NA	ND
PMA	66±15%	86±4%	66±3%	ND

*mLN, E8 and PA22-2 were used at a concentration of 10 μg/ml to coat the wells. hLN was used at a concentration of 2 μg/ml. These concentrations were shown in preliminary experiments to induce maximal adhesion of mast cells under the conditions studies. Anti α6 antibody (clone GoH3) was used at 10 μg/ml except when the inhibition of adhesion to hLN or PA22-2 was studied, where anti-α6 was used at 1 μg/ml. These antibody concentrations were shown to induce maximal inhibition in each case. In all experiments, rat IgG2a and anti-CD44 antibody were used as control. Neither inhibited mast cell adhesion to any of the peptides and proteins tested. Data is expressed as mean±SEM, $n=3$. ND=not determined, NA=not applicable because there is no spontaneous adhesion.

the adhesion assay. Eleven–67% of PMA-induced adhesion to murine laminin is also blocked by anti-α6 antibody. This data on inhibition of adhesion with anti-α6 antibody is consistent with FACS and immunoprecipitation data showing that α6 is uniformly expressed on all mast-cell populations (Figs 3 and 4d). The same anti-α6 antibody also inhibits the adhesion of mast cells to E8 (35–66%), where an α6 binding site is located.[21] Our experiments also show that PA22-2 is a candidate for an active site in the adhesive interaction between α6 on mast cells and laminin. A recent paper identified two other areas of E8, with a similar potential interaction with α6 integrin.[22]

It is clear that not all adhesion of mast cells to laminin is explained by an α6 containing integrin because not all adhesion to laminin may be blocked by anti-α6 antibody. Other surface receptors may thus also play a role in the interaction of mast cells with laminin. No α1 integrin could be detected on mast cells and α2 could be detected only on BMCMC and, therefore, could not account for C57 adhesion to laminin. The demonstration that message is found in some mast cell populations by RT–PCR for α3 integrin (Fig. 4a) also suggests that this receptor may be involved in mast cell adhesion to laminin in some situations. Furthermore, a 67 000 MW laminin receptor has been shown to be expressed on mast cells and antibodies against this receptor partially inhibit the adhesion of mast cells to mouse laminin.[23] The active site for adhesion to the 67 000 MW receptor belongs to the B1 chain of laminin[24] so this receptor cannot mediate adhesion to E8. The 67 000 MW receptor is a more likely candidate for additional adhesion to mouse laminin than α3 integrin, as it has been shown that K562 cells transfected with α3 A cannot adhere to mouse laminin either spontaneously or after activation.[25] However, these transfected K562 cells could adhere to human placental

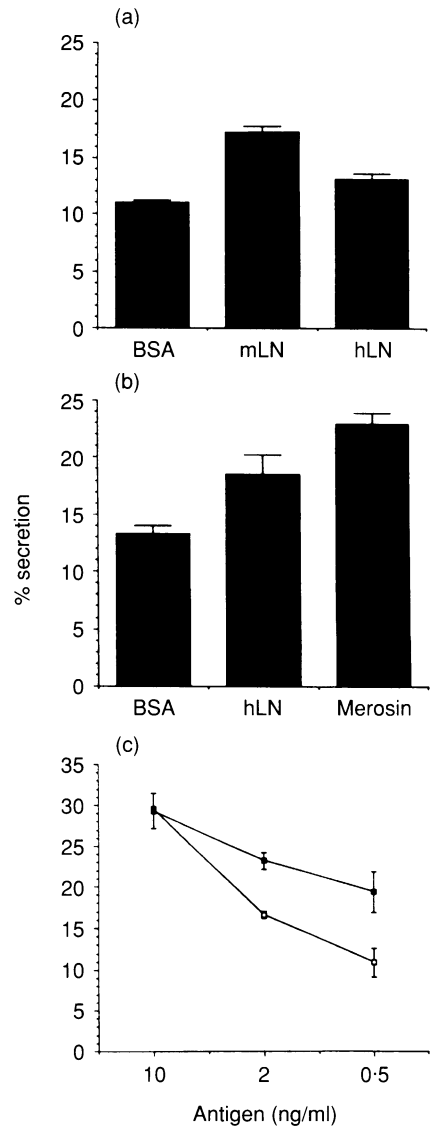

Figure 5. Mast cell secretion of β-hexosaminidase as altered by adhesion to laminin. (a) β-hexosaminidase secretion from C57 cells after activation through FcεRI aggregation with or without adhesion to mLN or hLN. Data is presented as mean±SEM, $n=3$. (b) β-hexosaminidase secretion from BMCMC after activation through FcεRI aggregation with or without adhesion to hLN or merosin. Data is presented as mean±SEM, $n=3$. (c) β-hexosaminidase secretion from BMCMC after FcεRI crosslinking with different concentrations of antigen without adhesion (open circles) or after adhesion (closed circles) to merosin. Data is presented as mean±SEM, $n=2$. (All experiments were done in triplicate.)

laminin. Therefore, this receptor could be partially responsible for the residual stimulated adhesion of mast cells to human laminin after partial inhibition with anti-α6 antibodies. Finally, αvβ3 that has been shown to be expressed on BMCMC[5] and on *in vitro* developed human mast cells,[20] could also play a role in this interaction. The participation of more than one receptor in the interaction of cells with laminin is well described. Antibodies against the 67 000 MW laminin receptor inhibit 30% of the adhesion of hepatocytes to laminin.[26] Human thymocyte adhesion to laminin is inhibited completely

by a mixture of α3 and α6 antibodies, but only partially by each antibody alone.[13] Integrins α3 and α6 are also responsible for 100% of the adhesion of the human leukaemic mast-cell line HMC-1 to human placental laminin.[27]

Cell adhesion to extracellular matrix proteins has been shown to affect cellular function[28] including cytokine production,[29] metalloproteinase gene expression[30] and mediator secretion;[31–32] Data presented in this paper demonstrate an increase in mast cell granule enzyme release following FcεRI mediated mast cell activation when the cells were adherent to different isoforms of laminin (Fig. 5). This synergistic effect was evident when suboptimal concentrations of antigen were used to induce secretion from mast cells but adhesion to laminin did not increase the highest amount of β-hexosaminidase released from mast cells with optimal antigen concentration. This synergistic effect is at least partially mediated through the interaction of laminin with α6 integrin on the surface of mast cells. This effect of laminin on mast cell secretion might have important biological consequences in cases where mast cells are found in areas with increased amounts of extracellular matrix (ECM) proteins and specifically laminin, such as in cases of fibrosis.

In summary, it is clear that mast cells adhere to various laminin isoforms following physiological stimulation. This adhesive interaction is in large part mediated by α6 containing integrins and results in an enhanced release of granule constituents following FcεRI mediated cell activation. These data also suggest that adhesive interactions between mast cells and laminin contribute to the regulation of mast-cell responsiveness within tissues.

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