

Induction of mast cell interactions with blood vessel wall components by direct contact with intact T cells or T cell membranes *in vitro*

A. Brill*, D. Baram†, U. Sela‡, P. Salamon†, Y. A. Mekori† and R. Hershkovich‡

*Hematology Department, Hadassah Medical Center, Jerusalem, Israel, †Department of Internal Medicine, Sapir Medical Center, Kfar Saba, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel and ‡Department of Internal Medicine D, Assaf Harofe Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Summary

Background Mast cells exert profound pleiotropic effects on immune cell reactions at inflammatory sites, where they are most likely influenced not only by the extracellular matrix (ECM) and inflammatory mediators but also by the proximity of activated T lymphocytes. We recently reported that activated T cells induce mast cell degranulation with the release of TNF- α , and that this activation pathway is mediated by lymphocyte function-associated antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1) binding.

Objective To determine how this contact between the two cell types can modulate mast cell behaviour in an inflammatory milieu by examining the adhesion of mast cells to endothelial cells and ECM ligands in an integrin-dependent manner.

Methods Human mast cells (HMC-1) were co-cultured with resting or activated T cells followed by testing their adhesion to endothelial cell and ECM ligands, stromal derived factor-1 α (SDF-1 α)-induced migration, and western blotting.

Results Co-culturing HMC-1 with activated, but not with resting T cells resulted in marked stimulation of mast cell adhesion to vascular cell adhesion molecule-1 and ICAM-1 in a very late antigen-4- and LFA-1-dependent fashion. In addition, activated T cells or T cell membranes promoted HMC-1 adhesion to fibronectin (FN) and laminin. This effect was accompanied by the phosphorylation of extracellular regulated kinase and p38, but not of c-Jun N-terminal kinase. Importantly, the adhesive property of mast cells depended exclusively on the direct contact between the two cell types, since neither supernatants from activated T cells nor separation of the two cell populations with a porous membrane affected mast cell adhesion to FN. Furthermore, similar results were obtained when mast cells were incubated with purified membranes from activated T cells. These results suggest that, in addition to stimulating mast cell degranulation, the proximity of activated T lymphocytes to mast cells can mediate the adhesion of mast cell precursors to the endothelial ligands and ECM. Activated T cells also stimulated SDF-1 α -induced mast cell migration.

Conclusion This symbiotic relationship between the two types of immune cells may serve to direct mast cells to specific sites of inflammation where their effector functions are required.

Keywords adhesion, ERK, mast cells, migration, p38, T cells

Submitted 25 November 2003; revised 16 June 2004; accepted 13 July 2004

Introduction

Mast cells are implicated in a variety of allergic and non-allergic responses through their ability to degranulate upon activation, consequently releasing cytoplasmic stores of cytokines, proteases, histamine, and heparin. Mast cell progenitors invade from the peripheral circulation into distinct tissues where they differentiate into mature resident cells; however, mast cell hyperplasia only occurs under certain inflammatory conditions [1]. The role of mast cells in exerting multiple responses in their resident tissue, via the release of soluble mediators, may largely depend on their vicinity to

vascular endothelial cells, as well as their interactions with extracellular matrix (ECM) molecules within connective tissue. Mast cell adhesion to the endothelium and ECM constitutes an essential process in the cascade of events, leading to cellular transmigration and localization at specific inflammatory sites. Furthermore, mast cell adhesion to the major ECM component, fibronectin (FN), via cell surface integrins, very late antigen (VLA)-4, and VLA-5 has been shown to enhance Fc ϵ RI-mediated degranulation [2]. The regulation of mast cell adhesion has also been ascribed to Fc ϵ RI cross-linking and different soluble or ECM-bound mediators, including *c-kit* ligand and serum amyloid A [3, 4]. However, the role of other inflammatory cells, such as T lymphocytes, in providing pro-adhesive or other stimulatory signals to mast cells via direct cell-to-cell contact has not yet been elucidated.

Correspondence: Dr A. Brill, Hematology Department, Hadassah Medical Center, Jerusalem, 91120, Israel. E-mail: brill@hadassah.org.il

Intercellular communication via cell–cell interactions is probably a significant mechanism governing immune cell behaviour at inflamed sites, where several cell types accumulate. Previously, we examined the effect of direct contact between mast cells and T lymphocytes regarding mast cell degranulation [5]. In addition, we recently described an autocrine TNF- α -mediated mechanism regulating secretion of matrix metalloproteinase-9 from human mast cell (HMC) after their contact with membranes of activated T cells [6]. As a result, a functional relationship between these two immune cell types has been proposed, since recent studies have revealed mast cells in close interaction with T cells within inflamed allergic tissues [7]. It seems likely that such a relationship may provide bidirectional signals, since T cells can mediate mast cell proliferation and activation, and the granular products of activated mast cells may promote T cell-mediated inflammatory reactions [8, 9]. Our previous findings that cell-to-cell contact between mast cells and activated T cells induces mast cell degranulation via intercellular adhesion molecule-1 (ICAM-1)/lymphocyte function-associated antigen-1 (LFA-1) provided further evidence of a putative relationship between the two cells [5]. Here, we demonstrate that mast cell–T cell contact up-regulates mast cell adhesion to the receptors of the endothelial cells as well as adhesion to the ECM ligands such as FN and laminin (LN), accompanied by mitogen-activated protein (MAP) kinase activation. These results suggest that the interactions between mast cells and activated T lymphocytes may promote mast cell binding and localization within target tissues, where their release of soluble proteases may in turn regulate ECM degradation during inflammation and tissue remodelling.

Materials and methods

Materials

The following antibodies were purchased from Serotec (Oxford, UK): anti-VLA-4 monoclonal antibody (mAb), anti-LFA-1 mAb, anti-human VLA-5 mAb, anti-human VLA-6 mAb, and anti-human ICAM-1 mAb. Anti-human CD3 mAb (clone OKT-3) was kindly provided by Prof. Z. Eshhar (The Weizmann Institute of Science, Israel). mAbs anti-pERK (extracellular regulated kinase; clone B180), anti-pP38, and anti-pJNK (c-Jun N-terminal kinase) were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). Other materials included recombinant human ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) (R&D systems, Minneapolis, MN, USA), purified human LN (Sigma Chemical Co., Rehovot, Israel), purified human FN (Chemicon, Temecula, CA, USA), phorbol myristate acetate (PMA) (Sigma Chemical Co.), and Na⁵¹Cr (Dupont-NEN, Boston, MA, USA). PD098059 was kindly supplied by Prof. R. Seger (The Weizmann Institute of Science). Cytochalasin D was purchased from Sigma Chemical Co. Reagents for cell culture were purchased from Kibbutz Beit Haemek (Israel) unless stated otherwise.

Cell cultures

HMC-1, human mast cell leukaemia cells, were maintained in RPMI supplemented with 10% FCS, 2 mM L-glutamine, and 1% penicillin–streptomycin. To study the effects of co-culture with

activated T cells, human peripheral blood T lymphocytes were isolated from healthy donors as previously described [10]. Briefly, the mononuclear cells were isolated on Ficoll gradients, and then washed and incubated for 2 h, 37 °C, 7.5% CO₂ in a humidified incubator. Non-adherent cells were collected and incubated on nylon wool columns (Novamed Ltd, Jerusalem, Israel). Unbound cells were eluted by extensive washing with phosphate-buffered saline (PBS), and the resulting cell population was >92% T lymphocytes. Co-cultures of HMC-1 cells and freshly isolated T lymphocytes (at a 1:1 ratio) were incubated in adhesion media (RPMI supplemented with 2 mM L-glutamine, 1% penicillin/streptomycin, 1% sodium pyruvate, and 0.1% bovine serum albumin (BSA)) in 96-well cluster plates or in 24-well cluster plates containing 6.5-mm chambers with Transwell polycarbonate membranes (5- μ m pores) for 18 h at a 1:1 ratio.

T cell activation

Freshly isolated human peripheral blood T cells (1×10^6 /mL) were activated with PMA (50 ng/mL) for 1 h, followed by extensive washing (twice) with RPMI-1640. Next, washed T cells were resuspended in RPMI-1640 supplemented with L-glutamine, penicillin–streptomycin–nystatin, and 0.1% BSA and then used for co-culture with HMC-1.

Isolation of T cell membranes

Resting or PMA-activated cells (2×10^8) were washed three times with PBS (8 min, $200 \times g$) and resuspended at 10^7 cells/mL in ice-cold TKMS lysis buffer, comprising 50 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl₂, 0.25 M sucrose, 1 mM phenylmethyl-sulphonyl fluoride (PMSF), and a cocktail of protease inhibitors (Boehringer Mannheim, Mannheim, Germany). Alternatively, T cells (1×10^6 /mL) were placed on a tissue culture plate pre-coated with anti-CD3 mAb (1 μ g/mL, 60 min, 37 °C) for 1 h. The plate was then centrifuged ($160 \times g$, 5 min), and the cell medium was replaced by the described buffer. In both cases, the cells were kept on ice for 20 min, and lysed by five cycles of freezing and thawing in liquid nitrogen, until no living cells could be observed by trypan blue exclusion. The cells were then centrifuged at $800 \times g$ for 5 min at 4 °C. Next, the supernatants were collected and centrifuged for 60 min at $100\,000 \times g$, 4 °C. Finally, the pellets were suspended in PBS, and stored at –70 °C.

Cell adhesion assays

Mast cell adhesion to endothelial cells or ECM ligands was determined as previously described [10]. Flat-bottomed wells in microtitre dishes were pre-coated with FN (10 μ g/mL), LN (10 μ g/mL), VCAM-1 (10 μ g/mL), or ICAM-1 (10 μ g/mL) for 1 h at 37 °C. Alternatively, 24-well culture plates were coated with the ECM ligands for the Transwell membrane experiments in which the two cell populations were separated. After extensive washings, the wells were blocked with 0.1% BSA. ⁵¹Cr-labelled mast cells (10^5 cells/100 μ L RPMI-1640 containing 0.1% BSA) were added to the wells either alone or simultaneously with an equal number of unlabelled T cells, and the plates were incubated at 37 °C for 1 h. In certain wells, namely with Arg-Gly-Asp (RGD) peptide (100 μ g/mL) or Arg-Gly-Glu-Ser (RGES) peptide (100 μ g/mL), blocking mAbs specific for the integrins VLA-4, VLA-5, VLA-6, CD29, or

LFA-1 (all in concentration of 5 µg/mL) were added together with the cells. In some samples, the cells were pre-incubated with cytochalasin D (2 µM) 1 h before the adhesion experiment. Non-adherent cells were removed by several washes with PBS, and adherent cells were lysed with 1 M NaOH/0.1% Triton X-100, and then radioactivity was measured in a γ -counter (Packard, Albertville, MN, USA). The results are presented as percentage of adhered cells to the total cell radioactivity.

Chemotaxis assays The migration of HMC-1 was examined in a 48-well chemotaxis micro-chamber (6.5-mm diameter; Corning, New York, USA), as previously described [10]. Briefly, the two compartments of the micro-chambers were separated by an FN-coated (1.25 µg/well) polycarbonate filter (5-µm pore size; Osmonics Proteins Products, Livemore, CA, USA). To induce HMC-1 chemotaxis, SDF-1 α (100 ng/mL) was added to the lower wells, and the HMC-1 (2×10^5 [^{51}Cr]-radiolabelled cells in a 100 µL/well) in RPMI (containing 0.1% BSA) were placed in the upper chambers. After the assay was completed (3 h, 37 °C, 7% CO₂), the filters were removed, and the number of migrating HMC-1 was determined by the level of radioactivity present in the lower wells. The control wells contained radioactivity of the cells that passed without a chemoattractant.

Analysis of protein phosphorylation by western blotting After co-incubation of mast cells with T cell membranes, the HMC-1 were lysed (30 min, 4 °C) in a buffer containing 25 mM Tris (pH 7.5), 1% Triton X-100, 0.5 mM EDTA, 150 mM NaCl, 10 mM NaF, 10 µg/mL leupeptin, 10 µg/mL pepstatin, 200 µg/mL PMSF, and a 1:100 phosphatase inhibitor cocktail. Next, the lysates were centrifuged, the solubilized proteins were separated under SDS-PAGE conditions, and then transferred to a cellulose membrane (Schleicher & Schuell, Dassel, Germany) at 90 V for 1 h. After blocking the membranes with 5% milk (2 h, 22 °C) diluted in Tris-buffered saline containing Tween (20 mM Tris (pH 7.5), 135 mM NaCl, 0.1% Tween-20), the membranes were incubated with mAb anti-pERK, anti-pP38, or anti-pJNK (18 h, 4 °C). Detection was performed using a horseradish peroxidase-conjugated secondary Ab (1 h, 20 °C), followed by visualization using an enhanced chemiluminescent system. All the bands were analysed densitometrically using the NIH 1.62 Image Program. Then, the same membranes were stripped, and the level of total protein (ERK) loaded was evaluated by blotting the membranes with anti-total ERK mAb. The level of phosphorylation, in percent, was calculated as follows: (the optical density (OD) of a given protein band of pERK/the corresponding OD of tERK) $\times$ 100. The amount of pERK in the treated HMC-1 was calculated according to the level of ERK phosphorylation in untreated cells (in the absence of adhesive substrates), which was considered as 100%.

Results

Co-culture of mast and T cells augments mast cell adhesion to endothelial cell ligands

In order to study whether mast cell–T cell contact affects the former cell's adhesion to endothelium, we performed adhe-

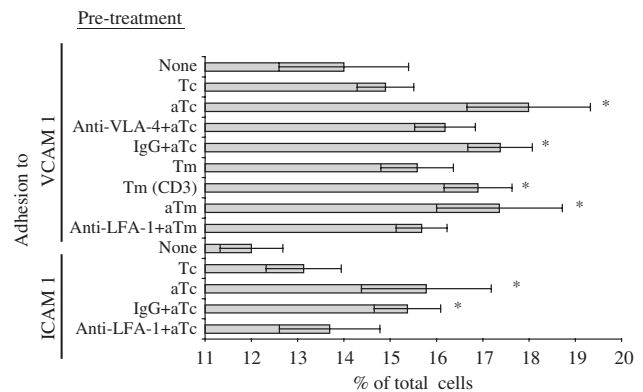


Fig. 1. Contact with activated T cells or T cell membranes stimulates human mast cells (HMC-1) adhesion to endothelial cell ligands. ^{51}Cr -labelled HMC-1 cells were co-incubated with activated or non-activated human T cells or T cell membranes for 18 h, after which the cells were placed into the wells pre-coated with either intercellular adhesion molecule-1 (ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1). In certain wells, either neutralizing monoclonal antibodies (mAbs) against very late antigen (VLA)-4 or lymphocyte function-associated antigen-1 (LFA)-1 (5 µg/mL) or irrelevant control IgG was added. The system was incubated for 1 h (37 °C), the wells were washed, and the remaining HMC-1 was lysed with 2% SDS solution. The radioactivity of the lysate was measured, which reflected the level of cell adherence. Untreated HMC-1 served as a negative control. The results were calculated as percentage of total cell radioactivity, and presented as the mean $\pm$ SD. Tc, T cells (non-activated); aTc, phorbol myristate acetate (PMA)-activated T cells; Tm, T cell (non-activated) membranes; aTm, PMA-activated T cell membranes; Tm (CD3), membranes of T cells activated with anti-CD3 mAb. The mean $\pm$ SD of four independent experiments performed in quadruplicate. *Statistically significant difference vs. control.

sion assays using purified endothelial ligands. Briefly, co-cultures of radiolabelled mast cells and unlabelled T cells were incubated on plates pre-coated with recombinant VCAM-1 or ICAM-1, and then the percentage of mast cell adhesion was measured after 1 h. As shown in Fig. 1, co-culturing of mast cells with pre-activated T cells resulted in a marked activation of the mast cell adhesion to VCAM-1 and ICAM-1. The enhancement of adhesion also occurred after co-incubation of mast cells with membranes of activated T cells. This finding proves that intracellular signalling in T cells, once having induced appropriate changes in the T cell membrane, is not involved in mediating the activatory effect on mast cells at the stage of the cell's co-incubation (Fig. 1). Such enhancement was inhibited by blocking antibodies to VLA-4 and LFA-1, when cells were incubated on VCAM-1, and to LFA-1, when the cells were incubated on ICAM-1. Mast cell attachment to plastic pre-coated with 0.1% BSA was always less than 5% (data not shown) of the total amount of cells. Thus, activated T cells provide a stimulatory signal to mast cells via cell-to-cell contact, which results in augmentation of mast cell–endothelial cell binding.

Activated T cells stimulate mast cell adhesion to extracellular matrix ligands

Mast cell adhesion to ECM constituents probably plays a major role in focusing and securing the cells' effector functions, such as degranulation, in inflamed tissue. Indeed, adhesion to FN via specific integrins was reported to enhance IgE receptor-mediated degranulation [2]. Since recent studies have also identified mast cell–T cell contact as an enhancer of Fc ϵ RI-dependent mast cell degranulation, and that such mast cell–T cell contact also enhances the adhesion of mast cells to

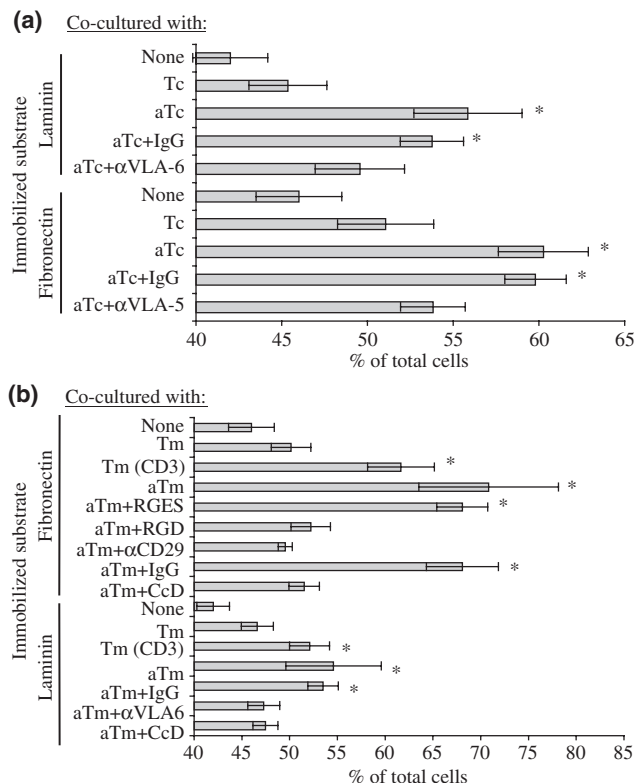


Fig. 2. Co-incubation with activated T cells or their membranes induces integrin-dependent adhesion of human mast cells (HMC-1) to extracellular matrix ligands. Radioactively labelled HMC-1 cells were co-incubated with activated or non-activated human T cells or T cell membranes for 18 h, and then the cells were seeded in the wells pre-coated with fibronectin or laminin. Integrin-blocking monoclonal antibodies (mAbs) (5 µg/mL), cytochalasin D, arginine-glycine-aspartic acid (RGD), peptide (100 µg/mL), or RGES peptide as a control (100 µg/mL) was added to the appropriate wells. After incubation (60 min, 37 °C), the non-adherent cells were removed, the adherent cells were lysed, and the percentage of HMC-1 that had adhered was determined. The results were calculated as percentage of total cell radioactivity and presented as the mean ± SD. Tc, T cells (non-activated); aTc, phorbol myristate acetate (PMA)-activated T cells; Tm, T cell (non-activated) membranes; aTm, membranes of T cells activated with PMA, Tm (CD3), membranes of T cells activated with anti-CD3 mAb. The mean ± SD of three independent experiments performed in quadruplicate. *Statistically significant difference vs. control.

the endothelial cell ligands (Fig. 1), we proposed that such contact also affects cell-matrix interactions. To investigate this hypothesis, we incubated co-cultures of activated T cells and HMC-1 cells on FN- or LN-coated microtitre plates. Adhesion of untreated HMC-1 to these ligands (in percentage of total cell radioactivity) was $46 \pm 2.39\%$ and $42 \pm 2.19\%$, respectively, which is less but comparable with the high spontaneous adhesion level of this cell line observed by others [11, 12]. We found that activated T cells and HMC-1 cells co-cultured resulted in increased mast cell adhesion to FN and LN by about 30% (Fig. 2a). This enhancement was dependent on HMC-1 cell surface integrins, as antibodies directed to the FN- and LN-binding $\beta 1$ -integrins, VLA-5 and VLA-6, blocked such adhesion (Fig. 2a). The signalling between interacting T cells and HMC-1 cells did not require intracellular T cell signalling or new protein synthesis by T cells, since isolated T cell membranes were sufficient to enhance mast cell adhesion (Fig. 2b). These results suggest that the binding between LFA-1, on activated T cell membranes, and ICAM-1, on intact mast cells, provides the

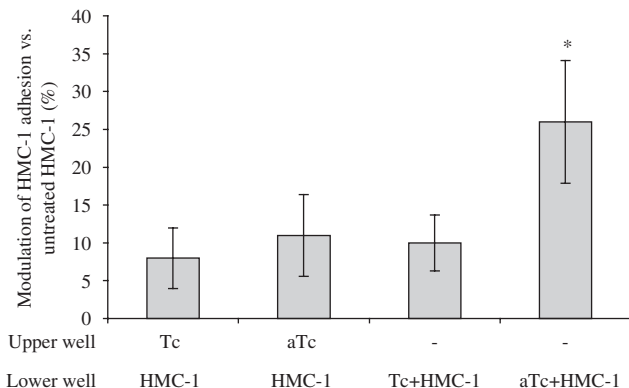


Fig. 3. Activated T cells exert their adhesion-activating effect on human mast cells (HMC-1) only in direct contact. Radioactively labelled HMC-1 cells were placed into the fibronectin-coated lower chamber of the Transwell system. Activated or non-activated human T cells were seeded either together with HMC-1, or in the upper chamber, isolated from the lower one by a permeable filter. After incubation (37 °C), the lower chamber was washed and the radioactivity of adhered HMC-1 was determined. The results are presented as adhesion change (%) in comparison with the adhesion of control untreated cells. Tc, T cells (non-activated); aTc, activated T cells. The mean ± SD of three independent experiments performed in duplicate. *Statistically significant difference vs. control.

necessary stimulus for mast cell adhesion to ECM components. The integrin dependence of the observed HMC-1 adhesion was further proven by its complete inhibition using a neutralizing mAb against integrin β -chain (CD29). In order to determine whether this pro-adhesive effect required rearrangement of the mast cell actin cytoskeleton machinery, we pre-treated HMC-1 cells with cytochalasin D. Treatment with this inhibitor of actin filament rearrangement completely inhibited the mast cell adhesion to FN and LN, enhanced by cell-to-cell contact with activated T cells (Fig. 2b).

We further examined the requirement for heterotypic aggregation between HMC-1 cells and activated T cells for up-regulation of mast cell adhesion, using the Transwell system. A permeable barrier consisting of a polycarbonate filter was used to separate resting or activated T cells in the upper compartment from HMC-1 cells in the lower FN-coated well. Alternatively, the two cell types were co-cultured in the lower compartment. HMC-1 cell adhesion was measured after 18 h. The results confirmed that augmentation of adhesion by activated T cells requires direct cell-cell contact and not secretion of soluble factors, since the placement of T cells in the upper compartment failed to induce mast cell adhesion to FN in the lower well, whereas co-culturing the cells in the lower well did induce this (Fig. 3). Therefore, we concluded that activated T cells confer a stimulus to mast cells that increases their adhesion to the ECM, and that this process is dependent on heterotypic aggregation and direct ligation of cell surface molecules.

Activated T cell membranes further stimulate mast cell migration towards SDF-1 α

Stromal derived factor-1 α (SDF-1 α) is a chemokine that is present in abundance in zones of inflammation. It is a potent chemoattractant for various kinds of cells including T and B lymphocytes and blood cell progenitors. Recent studies have demonstrated that the receptor for SDF-1 α (CXCR4) is expressed on mast cells [13] and that the SDF-1 α gradient is

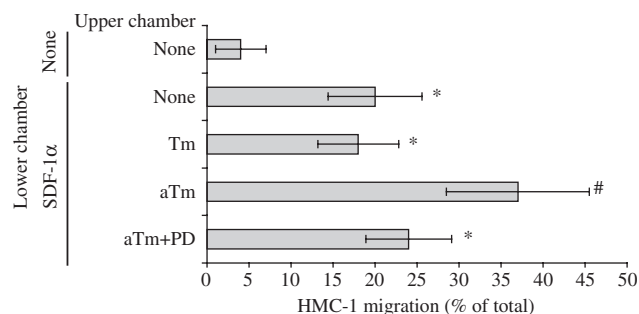


Fig. 4. Activated T cell membranes up-regulate human mast cells (HMC-1) migration towards SDF-1 α in an extracellular regulated kinase-dependent fashion. ^{51}Cr -labelled HMC-1 was placed into the upper chamber of a Transwell system, and SDF-1 α was placed into the lower chamber. In some experiments, HMC-1 was pre-incubated with an inhibitor of MAPK/ERK kinase, PD98059, for 30 min before the test. The radioactivity of the cells passing through the lower chamber was measured after a 3 h incubation (37 °C). The results were calculated as the percentage of total radioactivity of 2×10^5 cells, and presented as the mean $\pm$ SD. Tm, T cell (non-activated) membranes; aTm, activated T cell membranes. The mean $\pm$ SD of four independent experiments performed in duplicate. *Statistically significant difference vs. control, #statistically significant difference vs. SDF-1 α -induced migration of intact mast cells.

capable of inducing mast cells migration [13, 14]. Therefore, we studied the effect of the co-incubation of T cells with mast cells regarding the migration of the latter towards SDF-1 α gradients.

As seen in Fig. 4, the gradient of SDF-1 α induced a marked migration of mast cells, which corresponds to data in the literature. Interestingly, the preliminary contact of mast cells with non-activated T cells did not result in any modification of HMC-1 migration. However, the contact between mast cells and T cells that had been previously activated led to a marked activation of SDF-1 α -induced migration of HMC-1 (Fig. 4).

The migration-propagating effect of co-incubation of HMC-1 with T cells initiated the involvement of specific mechanisms responsible for cellular motility. One such molecule is ERK [15–17]. Therefore, we checked the effect of PD98059, which is an inhibitor of MAPK/ERK kinase, and as a result, of ERK, on HMC-1 migration. As seen in Fig. 4, treatment with PD98059 inhibited the migration of HMC-1 caused by contact with activated T cell membranes, down to the level of SDF-1 α only. Thus, ERK activation is necessary for the migration-propagating effect of HMC-1-T cell membrane contact.

Activated T cells stimulate the phosphorylation of extracellular regulated kinase and p38, but not c-Jun N-terminal kinase in mast cells

It is well established that MAP kinases participate in signalling pathways mediating the adhesive and migratory properties of different cells [18–20]. Therefore, we next investigated the effect of co-incubation of T cell membranes with mast cells on the phosphorylation of MAP kinases (ERK1, ERK2, and p38) in the mast cells. As shown in Fig. 5, co-incubation of mast cells with resting T cell membranes did not modify the level of ERK and p38 phosphorylation present in untreated mast cells. In contrast, when the T cells were pre-activated with PMA, incubation of their membranes with mast cells resulted in a marked up-regulation of the phosphorylation of ERK and p38. Blotting the same

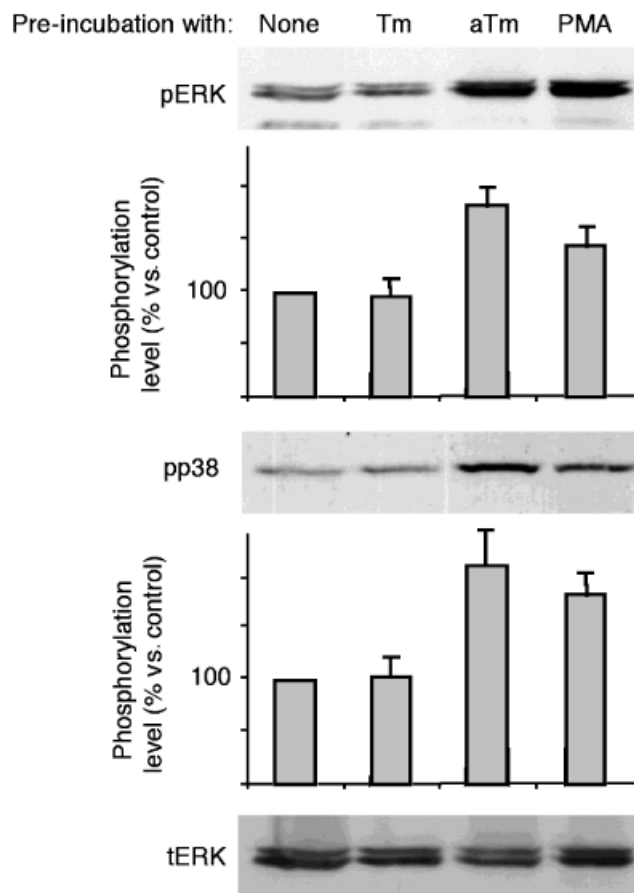


Fig. 5. Activated T cell membranes stimulate phosphorylation of extracellular regulated kinase (ERK) and p38 in human mast cells (HMC-1). HMC-1 cells (3×10^6 , in 250 μL of RPMI with 0.1% bovine serum albumin (BSA) per sample) were incubated (37 °C, 10 min) with membranes of activated or non-activated human T cells. HMC-1 activated with phorbol myristate acetate (PMA) (5 min) served as a control. The HMC-1 cells were then lysed in a buffer containing phosphatase inhibitors. The level of mitogen-activated protein kinase (MAPK) phosphorylation was analysed by western blotting using a specific monoclonal antibody against the phosphorylated form of ERK and p38. The SDS-PAGE represents two experiments out of four. Densitometric analysis is shown. The level of phosphorylation was calculated as follows: (the optical density (OD) of a given protein band of pERK/the corresponding OD of tERK) $\times$ 100. The amount of MAPK phosphorylation in the treated HMC-1 was calculated according to the level of pMAPK in untreated cells (in the absence of co-incubation with T cell membranes), which was considered as 100%. The densitometric histograms $\pm$ SD were calculated from the results of four different experiments.

membranes with an mAb against phosphorylated JNK, a kinase not so intimately involved in the processes of adhesion and migration, showed no activation of JNK phosphorylation in mast cells after co-incubation with activated T cell membranes (data not shown). Therefore, we concluded that the effects of T cell membranes on the adhesive properties of mast cells are accompanied by stimulation of the MAP kinases.

Discussion

The inflammatory milieu may contain several cell types, including monocytes/macrophages, B and T lymphocytes, neutrophils, and mast cells; the purpose of their respective

functions is to resolve inflammation and restore homeostasis. Although their effector functions may be specialized, these cells interact with each other and may thereby cause reciprocal modifications in their activities, such as the production of inflammatory cytokines [5] or changes in their activatory status [8, 9]. These changes in immune cell activities, stemming from direct intercellular contact, consequently affect important functions underlying the inflammatory reaction.

Clearly, mast and T cells have tight and symbiotic interactions, as evidenced during T cell-mediated inflammation [21]. Some such influences have been attributed to the biological effects of T cell-derived soluble mediators on mast cells. However, recent investigations of T cell–mast cell interactions have revealed a novel intercellular way of communication exclusively involving the binding of cell surface molecules. Mast cells have been shown to degranulate and produce TNF- α upon direct contact with activated T cells [22], a process regulated by the binding of LFA-1 and ICAM-1 [23]. Moreover, it was demonstrated that release of histamine from mast cells induced by direct contact with anti-CD3 mAb-activated T cells correlates with the kinetics of formation of aggregates of the mast cell and lymphocytes [24]. Considering that such T cell-dependent mast cell activation may also involve other changes in mast cell behaviour, we undertook the present study to determine whether mast cell adhesion to and interaction with the endothelial ligands and the ECM are also affected. Specifically, we examined HMC-1 adhesion to VCAM-1, ICAM-1, FN, and LN, as well as characterized the integrins mediating such adhesion. Furthermore, we explored the effects of T cells on the phosphorylation of the MAP kinases in mast cells. The data presented herein define multiple functions of activated T cells in modulating mast cell behaviour within inflamed tissues, namely the enhancement of adhesion to endothelial cells and ECM ligands. Collectively, the following functions elicited by mast cell–T cell interactions probably represent critical steps in mast cell activity during inflammation: [1] interactions with vascular cells at the inflamed site; [2] adhesion to the ECM components surrounding inflamed tissues; and [3] degradation and disruption of ECM structural integrity. The latter point is based on our previous findings [5].

Mechanisms mediating T cell effects on mast cells after cell-to-cell contact remain to be elucidated, although it is known that activated lymphocytes may affect mast cells at the genome level [25]. In the present work, we also showed that direct contact of the mast cells with T lymphocyte membranes results in phosphorylation of ERK and p38. The MAP kinases play an important role in the signalling in mast cells. For example, ERK1 and ERK2 become activated following the cross-linking of Fc ϵ RI [26], and the activation of ERK is important for the release of arachidonic acid metabolites [27]. Interestingly, in mast cells the pathway leading from Fc ϵ RI to ERK activation can bypass Ras and Raf-1 and go through other signalling molecules [28]. Other MAP kinase family members (p38 and JNK) can also be activated in mast cells, and it is possible that p38 negatively regulates the JNK pathway [27]. This fact may explain our findings that mast cell contact with T lymphocytes stimulates phosphorylation of p38, whereas JNK phosphorylation remains unchanged.

Note that among MAP kinases, ERK and p38 play an important role in the processes of cell adhesion and migration [18–20]. Thus, ERK is capable of phosphorylating myosin light chain kinase, leading to marked cytoskeletal changes pivotal for cell motility [29]. Moreover, in certain kinds of cells, ERK is translocated to the newly formed focal adhesion sites [30]. At the same time, JNK is more involved in the activation of various transcription factors, and not in mediating cell adhesion. In our work, we reported that mast cell–T cell membrane contact results in stimulation of ERK and p38, whereas JNK remains intact when the cell adhesion is modified. This fact indicates that the effect of cell–cell contact may affect particular signalling pathways mediating cell adhesion in a specific manner. Note, however, that the possibility that mast cell adhesion may be physically mediated to some extent by activated T cells and T cell membranes cannot be completely ruled out.

References

- 1 Ceponis A, Kontinen YT, Takagi M et al. Expression of stem cell factor (SCF) and SCF receptor (c-kit) in synovial membrane in arthritis: correlation with synovial mast cell hyperplasia and inflammation. *J Rheumatol* 1998; 25:2304–14.
- 2 Yasuda M, Hasunuma Y, Adachi H et al. Expression and function of fibronectin binding integrins on rat mast cells. *Int Immunol* 1995; 7:251–8.
- 3 Vosseller K, Stella G, Yee NS, Besmer P. c-kit receptor signaling through its phosphatidylinositol-3'-kinase-binding site and protein kinase C: role in mast cell enhancement of degranulation, adhesion, and membrane ruffling. *Mol Biol Cell* 1997; 8:909–22.
- 4 Hershkovitz R, Preciado-Patt L, Lider O et al. Extracellular matrix-anchored serum amyloid A preferentially induces mast cell adhesion. *Am J Physiol* 1997; 273:C179–87.
- 5 Vaday GG, Baram D, Salamon P, Drucker I, Hershkovitz R, Mekori YA. Cytokine production and matrix metalloproteinase (MMP)-9 release by human mast cells following cell-to-cell contact with activated T cells. *Isr Med Assoc J* 2000; 2:26.
- 6 Baram D, Vaday GG, Salamon P, Drucker I, Hershkovitz R, Mekori YA. Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF- α . *J Immunol* 2001; 167:4008–16.
- 7 Galli SJ. Complexity and redundancy in the pathogenesis of asthma: reassessing the roles of mast cells and T cells. *J Exp Med* 1997; 186:343–7.
- 8 Skokos D, Le Panse S, Villa I et al. Mast cell-dependent B and T lymphocyte activation is mediated by the secretion of immunologically active exosomes. *J Immunol* 2001; 166:868–76.
- 9 Villa I, Skokos D, Tkaczuk C et al. Capacity of mouse mast cells to prime T cells and to induce specific antibody responses *in vivo*. *Immunology* 2001; 102:165–72.
- 10 Ariel A, Yavin EJ, Hershkovitz R et al. IL-2 induces T cell adherence to extracellular matrix: inhibition of adherence and migration by IL-2 peptides generated by leukocyte elastase. *J Immunol* 1998; 161:2465–72.
- 11 Kruger-Krasagakes S, Grutzkau A, Baghramian R, Henz BM. Interactions of immature human mast cells with extracellular matrix: expression of specific adhesion receptors and their role in cell binding to matrix proteins. *J Invest Dermatol* 1996; 106:538–43.
- 12 Schoeler D, Grutzkau A, Henz BM, Kuchler J, Kruger-Krasagakes S. Interleukin-6 enhances whereas tumor necrosis factor alpha and interferons inhibit integrin expression and adhesion of human mast cells to extracellular matrix proteins. *J Invest Dermatol* 2003; 120:795–801.

- 13 Juremalm M, Hjertson M, Olsson N, Harvima I, Nilsson K, Nilsson G. The chemokine receptor CXCR4 is expressed within the mast cell lineage and its ligand stromal cell-derived factor-1 α acts as a mast cell chemotaxin. *Eur J Immunol* 2000; 30:3614–22.
- 14 Lin TJ, Issekutz TB, Marshall JS. Human mast cells transmigrate through human umbilical vein endothelial monolayers and selectively produce IL-8 in response to stromal cell-derived factor-1 α . *J Immunol* 2000; 165:211–20.
- 15 Kintscher U, Wakino S, Kim S, Fleck E, Hsueh WA, Law RE. Angiotensin II induces migration and Pyk2/paxillin phosphorylation of human monocytes. *Hypertension* 2001; 37:587–93.
- 16 Ho W, Uniyal S, Meakin SO, Morris VL, Chan BM. A differential role of extracellular signal-regulated kinase in stimulated PC12 pheochromocytoma cell movement. *Exp Cell Res* 2001; 263:254–64.
- 17 Bonacchi A, Romagnani P, Romanelli RG et al. Signal transduction by the chemokine receptor CXCR3: activation of Ras/ERK, Src, and phosphatidylinositol 3-kinase/Akt controls cell migration and proliferation in human vascular pericytes. *J Biol Chem* 2001; 276:9945–54.
- 18 Cho SY, Klemke RL. Extracellular-regulated kinase activation and CAS/Crk coupling regulate cell migration and suppress apoptosis during invasion of the extracellular matrix. *J Cell Biol* 2000; 149:223–36.
- 19 Paine E, Palmantier R, Akiyama SK, Olden K, Roberts JD. Arachidonic acid activates mitogen-activated protein (MAP) kinase-activated protein kinase 2 and mediates adhesion of a human breast carcinoma cell line to collagen type IV through a p38 MAP kinase-dependent pathway. *J Biol Chem* 2000; 275:11284–90.
- 20 Sano H, Zhu X, Sano A et al. Extracellular signal-regulated kinase 1/2-mediated phosphorylation of cytosolic phospholipase A2 is essential for human eosinophil adhesion to fibronectin. *J Immunol* 2001; 166:3515–21.
- 21 Malaviya R, Twisten NJ, Ross EA, Abraham SN, Pfeifer JD. Mast cells process bacterial Ags through a phagocytic route for class I MHC presentation to T cells. *J Immunol* 1996; 156:1490–6.
- 22 Bhattacharyya SP, Drucker I, Reshef T, Kirshenbaum AS, Metcalfe DD, Mekori YA. Activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact. *J Leukoc Biol* 1998; 63:337–41.
- 23 Inamura N, Mekori YA, Bhattacharyya SP, Bianchini PJ, Metcalfe DD. Induction and enhancement of Fc(ϵ)RI-dependent mast cell degranulation following coculture with activated T cells: dependency on ICAM-1- and leukocyte function-associated antigen (LFA)-1-mediated heterotypic aggregation. *J Immunol* 1998; 160:4026–33.
- 24 Mekori YA, Baram D. Heterotypic adhesion-induced mast cell activation: biologic relevance in the inflammatory context. *Mol Immunol* 2002; 38:1363–7.
- 25 Oh CK, Metcalfe DD. Activated lymphocytes induce promoter activity of the TCA3 gene in mast cells following cell-to-cell contact. *Biochem Biophys Res Commun* 1996; 221:510–4.
- 26 Suzuki H, Takei M, Yanagida M, Nakahata T, Kawakami T, Fukamachi H. Early and late events in Fc ϵ RI signal transduction in human cultured mast cells. *J Immunol* 1997; 159:5881–8.
- 27 Kimata M, Inagaki N, Kato T, Miura T, Serizawa I, Nagai H. Roles of mitogen-activated protein kinase pathways for mediator release from human cultured mast cells. *Biochem Pharmacol* 2000; 60:589–94.
- 28 Graham TE, Pfeiffer JR, Lee RJ et al. MEK and ERK activation in ras-disabled RBL-2H3 mast cells and novel roles for geranylgeranylated and farnesylated proteins in Fc ϵ RI-mediated signaling. *J Immunol* 1998; 161:6733–44.
- 29 Mansfield PJ, Shayman JA, Boxer LA. Regulation of polymorphonuclear leukocyte phagocytosis by myosin light chain kinase after activation of mitogen-activated protein kinase. *Blood* 2000; 95:2407–12.
- 30 Fincham VJ, James M, Frame MC, Winder SJ. Active ERK/MAP kinase is targeted to newly forming cell-matrix adhesions by integrin engagement and v-Src. *EMBO J* 2000; 19:2911–23.