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T Cell-Induced Mast Cell Activation: A Role for Microparticles Released from Activated T Cells

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Close physical proximity between mast cells and T cells has been demonstrated in several T cell-mediated inflammatory processes. However, the way by which mast cells are activated in these T cell-mediated immune responses has not been fully elucidated. We previously identified and characterized a novel mast cell activation pathway initiated by physical contact with activated T cells and showed that this pathway is associated with degranulation and cytokine release. In this study, we provide evidence that mast cells may also be activated by microparticles released from activated T cells that are considered miniature versions of a cell. Microparticles were isolated from supernatants of activated T cells by Centricon filtration or by high-speed centrifugation and identified by electron microscopy, flow cytometry (Annexin stain), and expression of the integrin LFA-1. Stimulated T cells were found to generate microparticles that induce degranulation and cytokine (IL-8 and oncostatin M) release from human mast cells. Mast cell activation by T cell microparticles involved the MAPK signaling pathway. The results were similar when mast cells were stimulated by activated fixed T cells or by whole membranes of the latter. This suggests that microparticles carry mast cell-activating factors similar to cells from which they originate. By releasing microparticles, T cells might convey surface molecules similar to those involved in the activation of mast cells by cellular contact. By extension, microparticles might affect the activity of mast cells, which are usually not in direct contact with T cells at the inflammatory site. *The Journal of Immunology*, 2010, 185: 4206–4212.

Most commonly known for their role in the elicitation of IgE-mediated allergic inflammation, mast cells have been implicated in a range of other nonallergic inflammatory processes. Observations, such as the close physical proximity between mast cells and T cells in inflamed tissues, and the capability of the former to release a wide range of immunomodulatory mediators have led investigators to propose a bidirectional functional relationship between these two cell populations (1, 2).

We previously reported on the effects of direct contact between mast cells and T lymphocytes on mast cell activation and mediator release (3–6). Mast cells were found to degranulate in response to direct contact with activated T cells or their membranes, as well to produce cytokines, such as TNF- α , IL-8, and oncostatin M (OSM) (4, 6). Moreover, studies using murine mast cells and PMA- or anti-CD3-activated T cells attributed the T cell-induced mast cell activation to interactions of surface molecules, such as ICAM-1 and lymphotoxin- β receptor, with their respective ligands (3, 7). Thus, direct contact between surface molecules on mast cells and on activated T cells was found to provide the stimulatory signal in mast cells necessary for degranulation and cytokine release, independent of intracellular T cell function and in the absence of

demonstrable soluble mediators (3–5). Indeed, separation of the two cell populations by a semipermeable porous membrane prevented this pathway of mast cell activation (3).

Based on the above information and because mast cells usually are not found in direct contact with T cells at the inflammatory sites, we hypothesized that mast cells may be activated by heterotypic adhesion to T cells, as well as by other components that may reside in the supernatants of activated T cells and are of high m.w., which does not allow their migration through the porous membranes. Possible candidates are membrane vesicles that are secreted by T cells (8, 9). Many reports described how portions of cell membranes can be transferred between cells, either after direct cell–cell contact or through the secretion of membrane vesicles. The functional consequences of such membrane transfers include the induction, amplification, and/or modulation of immune responses, as well as the acquisition of new functional properties by recipient cells (8, 9). Cells can generate membrane vesicles that are secreted into the extracellular space; such vesicles can form at the plasma membrane or in the lumen of internal compartments. Irrespective of their origin, these vesicles contain cytosol and expose the extracellular side of the membrane from which they form at their outer surface. Because their membrane orientation is the same as that of the donor cell, they can be considered miniature versions of a cell (8). Thus, membrane transfer is a mode of intercellular communication that may also involve T cell-induced mast cell activation within inflammatory sites in which both cell populations were shown to be involved, such as rheumatoid arthritis, Crohn's disease, and sarcoidosis (2).

As will be shown, activated T cells generate microparticles that induce degranulation and cytokine (IL-8 and OSM) release from human mast cells. The results were similar when mast cells were stimulated by activated fixed T cells or by whole membranes of the latter. This suggests that microparticles carry mast cell-activating factors similar to cells from which they originate. Thus, by releasing microparticles, T cells might convey surface molecules similar to those involved in the activation of mast cells by cellular

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Abbreviations used in this paper: OSM, oncostatin M; SCF, stem cell factor; sT, supernatants obtained from resting T cells; sT*, supernatants obtained from activated T cells; T*-m, membranes of activated T cells; T-mp, microparticles derived from resting T cells; T*-mp, microparticles derived from activated T cells.

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contact. By extension, microparticles might affect the activity of mast cells, which are usually not in direct contact with T cells at the inflammatory site.

Materials and Methods

Abs

Anti-phospho-ERK1/2 and ERK1/2 were purchased from Cell Signaling Technology (Danvers, MA). Anti-LFA-1/CD11a Ab was purchased from R&D Systems (Minneapolis, MN). HRP-conjugated secondary Abs were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Cell culture and reagents

Reagents for cell culture were purchased from Biological Industries (Beit Haemek, Israel). Jurkat T cell lymphoma cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 12.5 U/ml nystatin. Human peripheral blood T lymphocytes were maintained in Advanced RPMI 1640 (Invitrogen, Grand Island, NY), supplemented with 5% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 12.5 U/ml nystatin. The human LAD2 mast cells were established from bone marrow aspirates from a patient with mast cell sarcoma/leukemia and maintained in StemPro-34 SFM (Invitrogen) supplemented with 2 mM L-glutamine, 50 µg/ml streptomycin, 100 IU/ml penicillin, and 100 ng/ml recombinant human stem cell factor (SCF) (PeproTech, Rocky Hill, NJ), as previously described (10).

Primary cultured human cord blood mast cells were derived from human cord blood mononuclear cells in the presence of SCF, IL-6, and PGE₂ and used as previously described (6).

Isolation and activation of human peripheral blood T lymphocytes

Human peripheral blood T lymphocytes were isolated from healthy donors, as described previously (4). Briefly, the mononuclear cells were isolated by Ficoll-Hypaque density gradient, washed, and incubated for 60 min at 37°C on nylon wool columns (Uni-Sorb; Novamed, Jerusalem, Israel) in 5% CO₂ in a humidified incubator. Unbound cells were eluted by extensive washing with PBS, and the resulting cell population was >98% T lymphocytes. The freshly isolated human peripheral blood T lymphocytes (2 × 10⁶/ml) were stimulated or not for 60 min with 50 ng/ml PMA, as described previously (4), and cultured, following extensive washing, in RPMI 1640 supplemented with 5% FCS for 20 h at 37°C to isolate microparticles. In certain experiments, T cells were activated for 4–5 d at 37°C with anti-CD3/anti-CD28 mAb-coated beads, according to the manufacturer's instructions (Dynabeads; Invitrogen Dynal, Oslo, Norway). Cell viability was determined at the end of the incubation period by trypan blue staining. Viability was found to be >97% in all experiments.

Supernatants from activated or resting cells were collected and separated for the high m.w. fraction, as described below.

Supernatant fractionation

Supernatants of T cells, activated as described above, were collected after cell removal by centrifugation at 800 × g for 5 min and then centrifuged at 4500 × g for 5 min to discard large debris.

For separation of the high m.w. fraction, derived from supernatants of resting or activated T cells, acellular supernatants were subjected to 100,000-Da cut off Centricon (Vivaspin 2; Sartorius, Göttingen, Germany) and centrifuged at 4,000 × g for 10 min. After collection of the low molecular mass fraction (<100,000 Da) the Centricon was reversed, centrifuged at 4,000 × g for 2 min, and the high molecular mass fraction (>100,000 Da) was collected.

Isolation of microparticles

Microparticles were isolated from supernatants of resting or activated T cells, as described previously (11). Briefly, supernatants were obtained after cell removal by centrifugation at 800 × g for 5 min and then centrifuged at 4500 × g for 5 min to discard large debris. After additional centrifugation at 20,000 × g or ultracentrifugation (Beckman Ti70 rotor; Beckman Coulter, Brea, CA) at 100,000 × g for 60 min at 4°C, the microparticles were washed and resuspended in PBS. Microparticle protein concentration was measured at 280 nm using NanoDrop spectrophotometers (Thermo Scientific NanoDrop 1000; Thermo Fisher Scientific, Wilmington, DE). Protein quantity of microparticles was the same in all samples that were analyzed.

Mast cell activation

LAD2 mast cells or human cord blood mast cells were activated by incubation with cultured T cell supernatants, 50 µg/ml purified microparticles, or 20 µg/ml T cell-derived membranes, as previously described (4). For IgE-mediated activation, cells were sensitized overnight with 100 ng/ml human myeloma IgE-biotin (Calbiochem, Merck, Darmstadt, Germany) and then stimulated with 100 ng/ml streptavidin (Jackson ImmunoResearch Laboratories) (10).

β-hexosaminidase release

Activity of the secretory granule-associated enzyme β-hexosaminidase was determined as previously described (4). Results (mean ± SE) are expressed as the percentage of total β-hexosaminidase activity present in the cells.

Human cytokine assay

Supernatants obtained from the different culture conditions described above were examined for released IL-8 by a commercial ELISA kit, according to the manufacturer's instructions (Development kit; PeproTech Asia, Rehovot, Israel). OSM release was determined by using a commercial ELISA kit, as per the manufacturer's instructions (DuoSet; R&D Systems).

Analysis of microparticles by flow cytometry

Microparticles (50 µl) derived from resting or activated T cells were stained with Annexin V^{FLUO}, according to the manufacturer's instructions (MEBCYTO apoptosis kit; MBL, Nagoya, Japan) and analyzed by flow cytometry (Navios Flow Cytometers, Beckman Coulter), using Kaluza Flow cytometry-analysis software (Beckman Coulter), as previously described (11). An event-discrimination threshold was set on the side-scatter channel, and a size gate was set using 0.5- or 3-µm nonfluorescent beads

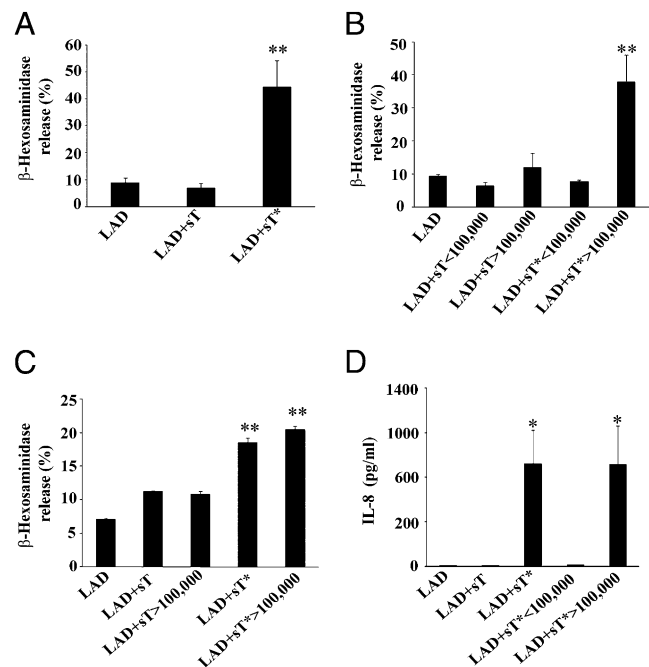


FIGURE 1. Supernatants of PMA-activated T cells stimulate β-hexosaminidase and IL-8 release from human mast cells. LAD2 human mast cells were incubated for 20 h with sT or sT* obtained from Jurkat T cells. **A**, After incubation, the supernatants were collected for the measurement of β-hexosaminidase release. sT or sT* obtained from Jurkat T cells (**B**, **D**) or human peripheral blood T lymphocytes (**C**) were subjected to separation by centrifugal filtration (Centricon) through 100,000-Da molecular mass cutoff filters. LAD2 cells were incubated with the low molecular mass fraction (<100,000 Da) or with the high molecular mass fraction (>100,000 Da) for 20 h, and β-hexosaminidase (**B**, **C**) or IL-8 (**D**) release was measured. Results are shown as mean ± SE of three independent experiments done in duplicate. **p* < 0.05; ***p* < 0.01. sT, supernatants obtained from resting T cells; sT*, supernatants obtained from activated T cells.

(FluoSpheres; Beckman Coulter) or 0.5- μ m red fluorescent beads (FluoSpheres Fluorescent Microspheres; Molecular Probes Invitrogen Detection Technologies, Paisley, U.K.).

Electron microscopy imaging

Microparticle samples were placed on a 400-mesh copper grid covered by carbon-stabilized Formvar film (Structure Probe, West Chester, PA). The mixture was allowed to stand for 1.5 min, excess fluid was removed, and the grids were negatively stained for 2 min with 10 μ l a 2% uranyl acetate solution. After removing the excess fluid, the samples were viewed using a JEOL 1200EX electron microscope (JEOL, Peabody, MA) operating at 80 kV.

SDS-PAGE and immunoblotting

Cellular extracts or microparticles were separated by SDS-PAGE using 10% or 15% polyacrylamide gels, transferred to nitrocellulose filters, and processed for immunoblotting, as previously described (4). Immunoreactive bands were visualized using the LAS-3000 imaging system (Fujifilm, Tokyo, Japan).

Statistical analysis

Results are represented as mean $\pm$ SE. Statistical analysis was performed using the unpaired Student *t* test. A *p* value <0.05 was considered statistically significant.

Results

The high m.w. fraction of supernatants from activated T cells induces mast cell activation

As previously reported, physical contact (heterotypic adhesion) is necessary for the stimulation of mast cells by activated T cells (3, 4). Because mast cells are usually not found in direct contact with T cells at the inflammatory site *in vivo*, we sought to investigate the involvement of other T cell-secreted factors in mast cell activation.

We first demonstrated that supernatants from PMA-activated T cells (75 ng/ml PMA for 1 h, followed by extensive washing)

induced mast cell degranulation, as measured by β -hexosaminidase release (Fig. 1). As can be seen in Fig. 1A, supernatants from resting T cells did not induce significant β -hexosaminidase release from LAD2 cells compared with control resting (nontreated) cells. However, a significant increase (~ 5 -fold) in β -hexosaminidase was found when LAD2 cells were cultured in the presence of supernatants obtained from activated T cells. The supernatants that were used to activate the mast cells did not contain any β -hexosaminidase (data not shown). To further characterize the factor in activated T cell supernatants, we used a method of m.w. separation. Thus, supernatants of PMA-activated T cells were subjected to separation by centrifugal filtration (Centricon). Only the high molecular mass supernatant fraction (molecular mass $>100,000$ Da) of activated, but not resting, T cells was able to induce β -hexosaminidase (Fig. 1B) or IL-8 (Fig. 1D) release from LAD2 mast cells. Of note, supernatants of the low molecular mass fraction ($<100,000$ Da) that were obtained from resting or activated T cells did not induce any significant mediator release. Similarly, supernatants of the high m.w. fraction that were obtained from PMA-activated, but not resting, freshly isolated peripheral blood T cells were also able to induce β -hexosaminidase (Fig. 1C) or OSM release (data not shown). Furthermore, high m.w. supernatants of freshly isolated peripheral blood T cells were able to induce significant degranulation and cytokine release when these cells were specifically activated by anti-CD3/anti-CD28-coated beads (Fig. 2). These results indicate the presence of a high m.w. T cell-derived mast cell secretagogue in supernatants of activated T cells. These results may also explain the previous finding whereby separation of the two cell populations by semipermeable barriers prevented mast cell activation (3). Because similar results were obtained by using peripheral blood T lymphocytes or Jurkat T cells, subsequent experiments were performed using Jurkat T cells.

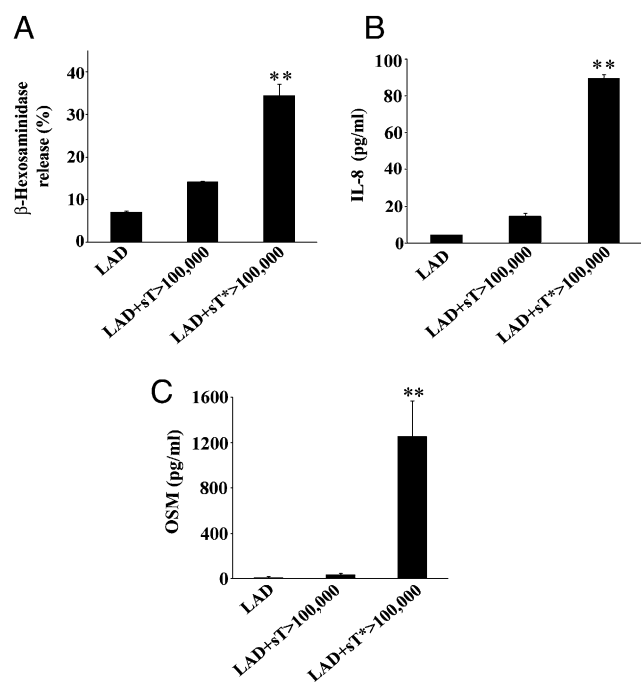


FIGURE 2. High m.w. supernatants of CD3/CD28-activated T cells stimulate β -hexosaminidase and cytokine release from human mast cells. LAD2 human mast cells were incubated for 20 h with the high molecular mass fraction ($>100,000$ Da) of supernatants obtained from resting or anti-CD3/CD28-activated human peripheral blood T lymphocytes. β -hexosaminidase (A), IL-8 (B), or OSM (C) release was measured at the end of the incubation period. Results are shown as mean $\pm$ SE of three independent experiments done in duplicate. ***p* <0.01 .

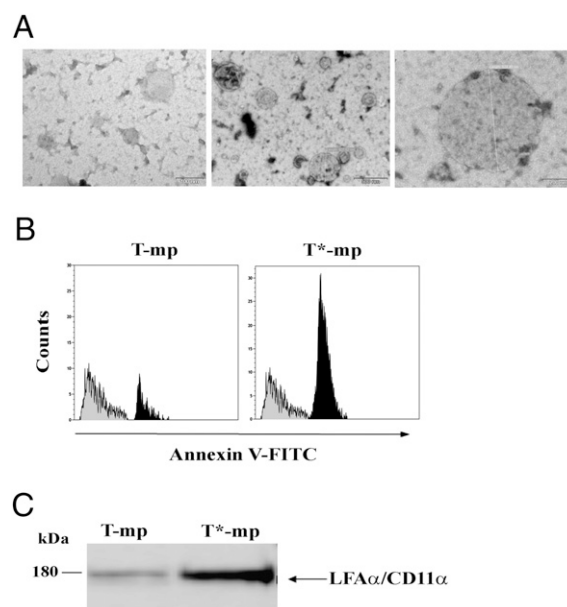


FIGURE 3. Characterization of activated T cell-derived microparticles. A, Microparticles obtained from resting (left panel) or activated (middle and right panels) T cells were examined by electron microscopy. Uranyl acetate, original magnification $\times 50,000$ or $\times 120,000$. B, Phosphatidylserine residues are exposed at the surface of microparticles obtained from T cells. Microparticles generated from activated or resting T cells were labeled with Annexin V-FITC and analyzed by flow cytometry. Fluorescence intensity (x-axis) versus microparticle count (y-axis) is shown. Gray graphs depict unlabeled microparticles; black graphs depict microparticles that were labeled with Annexin V-FITC. C, Expression of LFA-1/CD11a was measured by Western blotting of microparticles derived from sT or sT*.

Characterization of microparticles generated by activated T cells

Several reports described budding or shedding of large membrane vesicles (>100 nm in diameter) from plasma membrane of platelets, neutrophils, dendritic cells, and T cells. Such vesicles have been referred to as microvesicles or microparticles (8, 9, 11–13).

To investigate the presence of microparticles in the high m.w. supernatant fraction in our system, supernatants from PMA-treated Jurkat T cells were centrifuged by high-speed centrifugation, which has been shown to produce a pure preparation of microparticles (11, 12). Indeed, we were able to isolate microparticles from high m.w. supernatants of activated T cells and, to a much lesser degree, from resting T cells (3 $\mu\text{g}/\text{ml}$ compared with 1 $\mu\text{g}/\text{ml}$ of microparticle protein per 1×10^6 Jurkat cells, respectively). Ultrastructural analysis of microparticles isolated from resting or PMA-activated Jurkat T cells showed numerous membrane-coated round vesicles that ranged in size from 100–800 nm in diameter (Fig. 3A).

Microparticles have been shown to expose phosphatidylserine on their surface. Indeed, as can be seen in Fig. 3B, flow-cytometric analysis indicated that microparticles isolated from supernatants derived from activated or resting T cells bound Annexin V^{FLTC}, indicating that phosphatidylserine was exposed at their surface. Furthermore, the amount of microparticles isolated from activated T cells was higher (~4-fold) than the amount of microparticles isolated from resting T cells.

Microparticles also express adhesion molecules on their surface, which could favor their capture by recipient cells (8). LFA-1/

CD11a is expressed on the membrane of activated T cells and, therefore, represents a protein marker for microparticles generated from T cells (14). Western blot analysis of microparticles, obtained by high-speed centrifugation, demonstrated the presence of LFA-1/CD11a in samples derived from activated, but not resting, T cells when equal amounts of proteins were analyzed (Fig. 3C).

These data provide evidence that our high-speed centrifugation method resulted in the purification of microparticles with characteristics consistent with those previously described (8).

Microparticles derived from activated T cells induce mast cell degranulation

To assess whether microparticles from activated T cells can induce mast cell degranulation, purified microparticles were incubated for 20 h with human LAD2 cells. As shown in Fig. 4A, microparticles from resting T cells did not induce β -hexosaminidase release. However, a significant degranulation was found when LAD2 cells were incubated in the presence of microparticles obtained from activated T cells. This result was similar to the β -hexosaminidase release level obtained when LAD2 cells were incubated with purified membranes from activated T cells. Dose-response analysis with increased concentrations of microparticles from activated T cells revealed maximal release of β -hexosaminidase with 50 $\mu\text{g}/\text{ml}$ microparticles (Fig. 4B). Next, we analyzed the kinetics of degranulation induced by T cell microparticles. Thus, LAD2 cells were incubated with microparticles generated from activated T cells, with their membranes or activated by IgE cross-linking

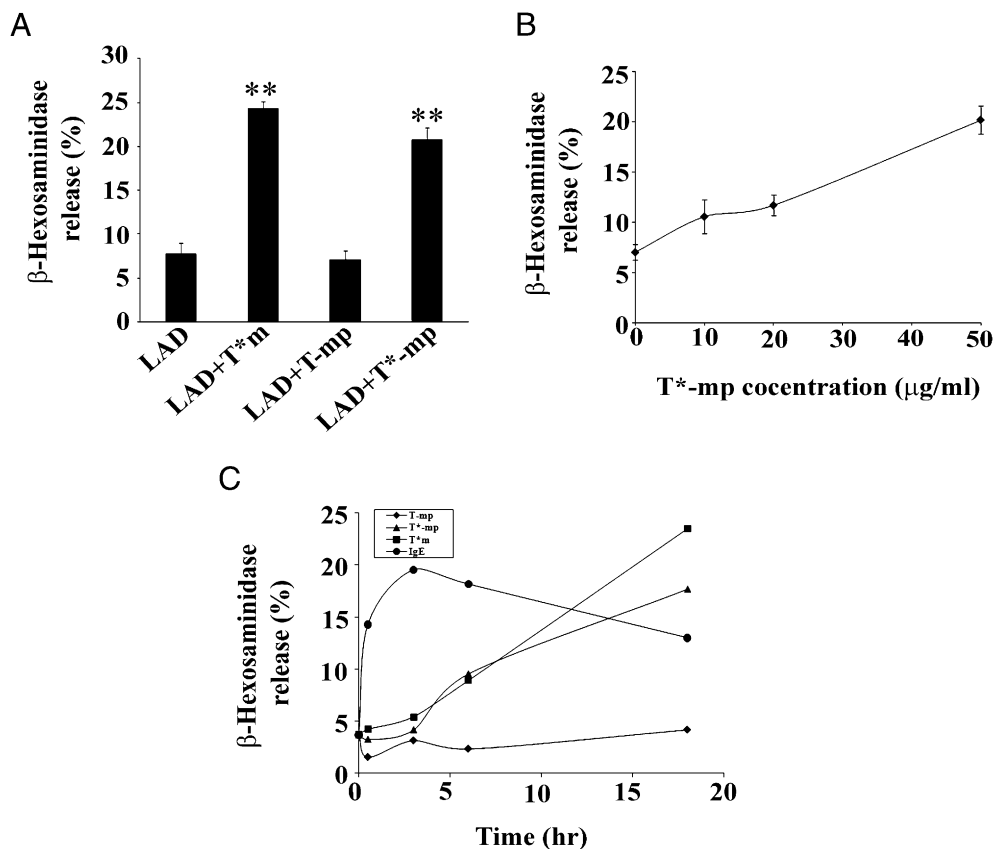


FIGURE 4. Microparticles generated by activated T cells induce β -hexosaminidase release from human mast cells. **A**, LAD2 mast cells were incubated with 50 $\mu\text{g}/\text{ml}$ of microparticles or 20 $\mu\text{g}/\text{ml}$ of membranes, both isolated from resting or activated T cells. Degranulation was assessed by β -hexosaminidase release. ** $p < 0.01$. **B**, Dose-response relationship of degranulation of LAD2 mast cells stimulated by increasing concentrations of microparticles generated from activated T cells, as measured at 20 h of incubation. **C**, Kinetics of degranulation induced by membranes (T*-m), IgE cross-linking, T*-mp, or T-mp. Results are shown as mean $\pm$ SE of five independent experiments done in duplicate. T*-m, membranes of activated T cells; T-mp, microparticles derived from resting T cells; T*-mp, microparticles derived from activated T cells.

for an incubation period of 18 h. Supernatants were collected at several time points for measurement of β -hexosaminidase (Fig. 4C). Microparticles obtained from activated T cells induced β -hexosaminidase release that was first detected at 4 h and peaked at 18 h of incubation. The same pattern of activation kinetics was noticed with membranes purified from activated T cells. In contrast, and as expected, IgE cross-linking induced a rapid-onset degranulation detected at 30 min, which peaked at 3 h after stimulation and then declined. It should also be noted that microparticles alone did not release any significant amount of β -hexosaminidase (data not shown). These results imply that similar mast cell-activation factors were present at the surface of microparticles and the cells from which they were generated.

Activated T cell microparticles induce cytokine release from human mast cells

In previous work, we showed that contact of mast cells with activated T cells or their membranes induced the release of cytokines, such as IL-8 and OSM (5, 6). To test whether microparticles generated from T cells induced the release of these cytokines, LAD2 cells were incubated for 24 h with purified T cell microparticles. As shown in Fig. 5, purified microparticles from resting T cells did not induce IL-8 (Fig. 5A) or OSM (Fig. 5B) release. However, a significant release of these cytokines (400 and 120 pg/ml of IL-8 and OSM, respectively) was measured when LAD2 cells were incubated in the presence of microparticles obtained from activated T cells. Experiments were also performed using mature (9–10-wk-old) human cord blood-derived primary mast cell cultures. Similar to our findings with the LAD2 cell line, a marked increase in IL-8 was observed in response to direct contact of these primary mast cell cultures with microparticles derived from supernatants of activated, but not resting, T cells (Fig. 5C).

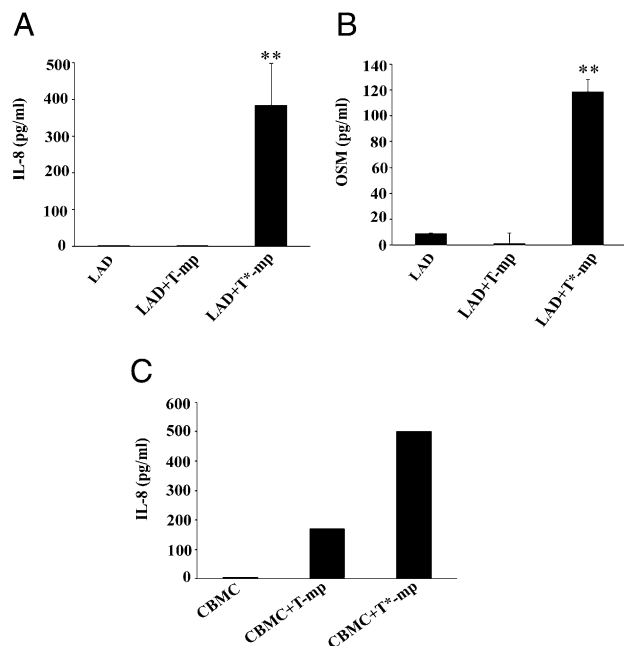


FIGURE 5. T cell-derived microparticles induce cytokine release from human mast cells. LAD2 mast cells were incubated with 50 μ g/ml T-mp or T*-mp for 20 h. Supernatants were collected and analyzed for IL-8 (A) or OSM (B) release by ELISA. Data represent mean $\pm$ SEM of three independent experiments done in duplicate. $**p < 0.01$. C, IL-8 release from human cord blood-derived mast cells incubated with 50 μ g/ml T-mp or T*-mp for 20 h.

These results suggest that activated T cell microparticles can induce human mast cell cytokine release in the same manner as observed with fixed T cells or their membranes (4, 5).

T cell-derived microparticles induce ERK phosphorylation in human mast cells

It was shown recently that stimulation of human mast cells by contact with activated T cells is associated with Ras activation and phosphorylation of the mitogen-activated MAPKs ERK (15, 16). Therefore, we investigated whether microparticles derived from activated T cells were also able to induce ERK activation. Thus, LAD2 cells were incubated with purified microparticles derived from resting or activated T cells or with their membranes for 5 min, and the level of phosphorylated ERK was recorded. As shown in Fig. 6A, microparticles derived from resting T cells induced a low level of ERK phosphorylation. In contrast, incubation of LAD2 cells with microparticles derived from activated T cells or their purified membranes induced high levels of ERK phosphorylation. These results demonstrate that mast cell activation by T cell microparticles may involve the MAPK signaling pathway.

We next investigated whether ERK signaling was involved in the release of β -hexosaminidase from LAD2 cells activated with T cell-derived microparticles. For this purpose, we pretreated the LAD2 cells with PD98059, a MAPK kinase/ERK inhibitor. As shown in Fig. 6B, a significant reduction ($\sim 60\%$) in β -hexosaminidase levels was observed, thus indicating a possible involvement of ERK in T cell microparticle-induced mast cell activation.

Discussion

It is now well accepted that mast cells are well engineered, highly developed, and critical to the induction of a number of specific and diverse immunological processes, in addition to allergic reactions. Referring specifically to T cell-mediated inflammatory processes, it was shown *in vivo* that mast cells were essential for appropriate cellular recruitment during T cell-induced cutaneous delayed-type hypersensitivity reactions (17). In this regard, T cells were found to

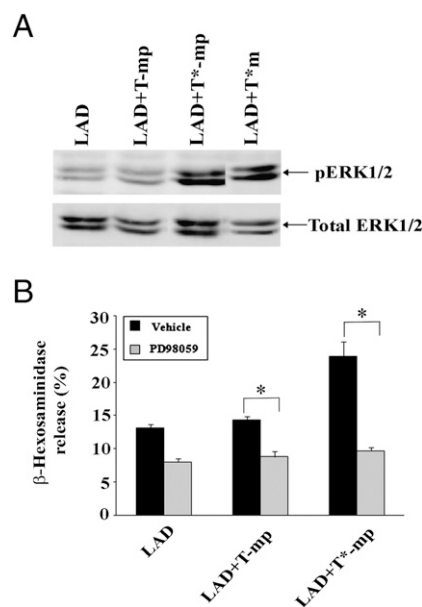


FIGURE 6. Effect of microparticles generated by activated T cells on the activity of ERK1/2. A, LAD2 mast cells were stimulated for 5 min with 50 μ g/ml T-mp or T*-mp. The phosphorylated ERK levels were analyzed by immunoblotting. B, LAD2 cells were stimulated overnight as indicated, with or without pretreatment with PD98059 (20 μ M). β -hexosaminidase release was measured at the end of the incubation period. $*p < 0.05$.

deliver signals that induced cytokine release from the neighboring mast cells. Although the nature of these signals *in vivo* has not been defined, it seems reasonable to suggest that a direct cross-talk between these two cell populations exists, because recent *in vitro* studies indicated that mast cells may be triggered to degranulate and release cytokines upon heterotypic adhesion to activated, but not resting, T cells (3–6). Our previous results and the present ones (Fig. 1) provide evidence that direct contact between cell-surface molecules on mast cells and on activated T cell membranes is sufficient to transduce the stimulatory signal in mast cells necessary for degranulation and cytokine release, independent of T cell intracellular function or production of cytokines or other mediators. However, when high m.w. fractions of supernatants of PMA or anti-CD3/CD28–stimulated T cells were investigated, a significant mast cell activation and mediator release were found (Figs. 1B–D, 2) indicating the presence of a mast cell secretagogue in this medium. The present study demonstrates that activated T cells generate microparticles that mimic the activation of mast cells by heterotypic adhesion to T cells (Figs. 4, 6).

Microparticles, also termed microvesicles, are small membrane-bound structures that are released from cells by exocytic budding of the membrane during activation or apoptosis. Their size ranges from 100 to 1000 nm, and they can be isolated at a sedimentation rate of 10,000 g. Microparticles express phosphatidylserine on their surface, which is normally confined to the inner leaflet and, therefore, could be captured by phosphatidylserine receptors. In addition to altered surface lipids, microparticles display surface markers from the parental cell from which they originate. Molecules, such as integrins and selectins, are expressed by microparticles, thus enabling their capture by the relevant receptors/ligands. The secretion of these microparticles is regulated by various stimuli that lead to an increased intracellular calcium level, which induces plasma membrane remodeling and vesicle shedding (8). Therefore, it can be concluded that we isolated microparticles from the high m.w. supernatants of activated T cells bearing all of the above characteristics (Fig. 3). Microparticles can serve as a rapid-release mechanism (i.e., to release cytokines or other biological transmitters) (9). The known emission of membrane-anchored receptors, adhesion molecules, enzymes, and signaling proteins via microparticles was recently complemented by the finding of functional mRNA and microRNA species in their cargo (9). Microparticles can transfer their content into various acceptor cells and, thereby, affect their properties. For example, and relevant to the current study, it was recently shown that ICAM-1–bearing exosomes derived from mature dendritic cells are captured by binding to its ligand LFA-1 on the surface of CD8⁺ and activated T cells (14). The adhesion pathway mediated by LFA-1 and its coligand ICAM-1 is one of the costimulatory pathways associated with T cells. It was demonstrated that LFA-1 (CD11/CD18)–dependent adhesion of T cells to ICAM-1 requires activation of protein kinase C by cross-linking cell-surface molecules, such as CD2 or CD3. Thus, similar to other cell systems, LFA-1–dependent adhesion of mast cells to T cells involves prior T cell activation. Indeed, it was shown that murine mast cell degranulation induced by coculture with activated T cells was dependent on ICAM-1/LFA-1–mediated heterotypic aggregation (3). Microparticles isolated from activated T cells in this study expressed LFA-1 on their surface (Fig. 3); however, introducing anti-LFA-1– and/or anti-ICAM-1–blocking Abs did not inhibit mast cell activation (data not shown), pointing to the possibility that other molecules in this cargo are responsible for transferring the activating stimulus. Possible candidate molecules that were shown to be expressed on activated T cells and to induce mast cell activation, such as lymphotoxin- β receptor (7) or OX40 (18), are under investigation in our laboratory.

Immune-activating properties of secreted particles have also been reported. Thus, microvesicles released by thrombin-activated platelets stimulated the proliferation, adhesion, and chemotaxis of hematopoietic cells (19), and endothelial cell-derived microparticles were found to induce plasmacytoid dendritic cell maturation and production of inflammatory cytokines (13). Microparticles occur abundantly in inflammatory processes in which mast cells were found to be involved, such as synovial fluid of rheumatoid arthritis (12). Activated T cell-derived microparticles induced matrix metalloproteinase and cytokine expression in synovial fibroblasts, thus pointing to a mechanism by which they can promote the destructive activity of these cells in rheumatoid arthritis.

Our present study revealed that activated T cell microparticles induced the production of IL-8 and OSM from LAD2 cells and human cord blood-derived primary mast cell cultures (Fig. 5). This is in accordance with our previous results that showed that contact of mast cells with activated T cells or their membranes induced the release of those cytokines (5, 6).

We recently showed that direct contact of human mast cells with T lymphocyte membranes resulted in the phosphorylation of the MAPK family (15, 16). The MAPKs are known to play an important role in mediator release in human mast cells after IgE–cross-linking or on activation by inducers, such as SCF and TNF- α (20, 21). In accordance with these findings, we were able to show that microparticle-induced degranulation is regulated by MAPKs, because it was abrogated on the addition of specific inhibitor to MAPK kinase/ERK inhibitor (Fig. 6). Thus, microparticles from activated T cells displayed comparable activity with cell membranes, suggesting that similar molecules are present in microparticles and at the surface of activated T cells. In addition to direct contact or juxtacrine effect on mast cells (4), T cells may contact and affect distant mast cells through the release of microparticles. Furthermore, because contact with T cell membranes or fixed activated T cells triggers degranulation and the production of several cytokines in mast cells (4–6), microparticles might remotely convey activating factors from T cells to mast cells and participate in the pathogenic mechanisms of T cell-induced inflammation. Our present findings are in agreement with a recent study by Scanu et al. (11) reporting that T cell-derived microparticles mimic cellular contact activation of monocytes.

In conclusion, this study demonstrated that microparticles generated by T cells carry similar molecules to the cells from which they originate because they are able to induce mast cell degranulation and cytokine release. Consequently, through the generation of microparticles, activated T cells may facilitate distant contact-mediated activation of mast cells that are not located in close physical proximity at the inflammatory sites. Blocking the release of microparticles and their interaction with mast cells, as well as downstream activation events, may serve as a therapeutic approach for T cell-mediated inflammatory processes in which mast cells are involved.

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Disclosures

The authors have no financial conflicts of interest.

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