T cell–derived microvesicles induce mast cell production of IL-24: Relevance to inflammatory skin diseases

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Background: It has recently been shown that microvesicles derived from activated T cells can stimulate human mast cells (MCs) to degranulate and release several cytokines.
Objective: The aim of this study was to characterize microvesicle-induced MC expression patterns. Through identification of unique cytokine and chemokine expression, we attempted to reveal pathogenetic roles for this pathway of MC activation.
Methods: T cell–derived microvesicles were labeled with PKH67 to allow visualization of their interaction with human MCs. Consequent gene expression profiling was studied by using a whole-genome microarray and analyzed for identification of cellular pathway clusters. Expression of 3 selected genes, chemokine (C-C motif) ligand 3 (*CCL3*), chemokine (C-C motif) ligand 7 (*CCL7*), and *IL24*, was validated by means of quantitative RT-PCR and specific ELISA. *IL24*, which has not been recognized heretofore in MCs, was also tested for its effect on keratinocyte signal transducer and activator of transcription 3 phosphorylation and for its presence in MCs in psoriatic skin lesions.
Results: Uptake and internalization of activated T cell–derived microvesicles into human MCs occurred within 24 hours. Microvesicles induced the upregulation of several clusters of genes, notably those that are cytokine related. Among these, *IL24* appeared to be a hallmark of microvesicle-induced activation. MC-derived IL-24, in turn, activates keratinocytes *in vitro*, as manifested by signal transducer and activator of transcription 3 (STAT3) phosphorylation, and is produced in MCs within psoriatic lesions.
Conclusion: Production of IL-24 is a unique feature of microvesicle-induced MC activation because its production by these cells has not been recognized thus far. We propose that this MC-derived cytokine might contribute to the pathologic findings in T cell–mediated skin inflammation. (J Allergy Clin Immunol 2014;133:217-24.)

Key words: *IL-24, mast cells, microvesicles, T cells*

Abbreviations used
CCL3: Chemokine (C-C motif) ligand 3
CCL7: Chemokine (C-C motif) ligand 7
ERK: Extracellular signal-regulated kinase
JNK: Jun N-terminal kinase
MAPK: Mitogen-activated protein kinase
MC: Mast cell
mvT: Microvesicles derived from resting T cells
mvT*: Microvesicles derived from activated T cells
STAT3: Signal transducer and activator of transcription 3

Mast cells (MCs) play a significant role in the establishment of innate and adaptive immune responses in addition to their role as pivotal effector cells in allergic responses. Close physical proximity between MCs and T cells in inflamed tissues have led investigators to propose a bidirectional interaction between these 2 cell populations.[1-3] Furthermore, morphologic studies have documented an increase in the local density and activation of MCs in T cell–mediated inflammatory processes, as observed in patients with rheumatoid arthritis, psoriasis, cutaneous delayed-type hypersensitivity reactions, graft-versus-host reactions, sarcoidosis, Crohn disease, and fibrosis.[3,4-6]

We have previously reported that direct contact with activated T lymphocytes or their membranes induced MC activation and mediator release.[7-9] Thus, direct contact between surface molecules on MCs and on activated T cells was found to provide the stimulatory signal necessary for degranulation and cytokine release independent of T-cell intracellular function and in the absence of demonstrable soluble mediators.

Moreover, MCs can also be activated by microvesicles released from T cells, thereby allowing response in the absence of contact with T cells at the site of inflammation. Microvesicles from activated, but not resting, T cells were found to induce degranulation and cytokine release from both human cord blood–derived MCs and human MC lines.[10] This finding suggests that microvesicles carry similar MC-activating factors, as do the cells from which they originate. Thus by shedding microvesicles, T cells convey activating surface molecules in a manner that does not require physical cell-cell contact.

In the present study we have characterized MC expression of inflammatory mediators after exposure to microvesicles derived from activated T cells (mvT*). Whole-genome expression profiling disclosed the production of several cytokines and chemokines that have been unknown thus far to be expressed in MCs. One of these mediators, IL-24, has been described as a potent keratinocyte activator in patients with inflammatory skin diseases.[11] Along this line, we have demonstrated that after treatment with T cell–derived microvesicles, MC-derived IL-24 induced phosphorylation of signal transducer and activator of transcription 3 (STAT3) in human

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keratinocytes. Finally, we show that IL-24 is expressed by MCs in psoriatic skin lesions. To the best of our knowledge, this is the first report of IL-24 in human MCs. On the basis of the previously reported effects of IL-24, the present study suggests that the production of IL-24 represents a link between T cell–induced MC activation and the pathogenesis of T cell–mediated inflammatory skin diseases in which MCs have been found to be involved.

METHODS

Antibodies and reagents

The following antibodies were used for this study: anti–phospho-STAT3 (Cell Signaling Technology, Danvers, Mass); anti–tubulin antibody (Sigma-Aldrich, St Louis, Mo); horseradish peroxidase–conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, Pa); phycoerythrin-CD117 (Beckman Coulter, Brea, Calif); and anti-human IL-24 antibody and normal goat IgG control (R&D Systems, Minneapolis, Minn). The inhibitors PD98059, curcumin, and cytochalasin D were purchased from Sigma-Aldrich; SB202190 was purchased from Calbiochem (San Diego, Calif); and Stattic was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif).

Cell culture

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 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 12.5 U/mL nystatin. The human LAD2 MCs were maintained in StemPro-34 SFM (GIBCO Invitrogen, Grand Island, NY) supplemented with 2 mmol/L L-glutamine, 50 μg/mL streptomycin, 100 IU/mL penicillin, and 100 ng/mL recombinant human stem cell factor (PeproTech, Rocky Hill, NJ), as previously described.[12] Primary cultured human cord blood MCs were derived from human cord blood mononuclear cells in the presence of stem cell factor, IL-6, and prostaglandin E_2 and used as previously described.[9]

The human keratinocyte cell line HaCaT was kindly provided by Dr Amiram Ravid (Tel Aviv University, Tel Aviv, Israel). Cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 12.5 U/mL nystatin.

Isolation of microvesicles

Microvesicles were isolated from supernatants of resting or activated T cells and identified as such, as described previously.[10,13] Briefly, supernatants were obtained after cell removal by means of centrifugation at 800*g* for 5 minutes and then centrifuged at 4500*g* for 5 minutes to discard large debris. After additional centrifugation at 20,000*g* for 60 minutes at 4°C, the microvesicles were washed with PBS by means of additional centrifugation at 20,000*g* for 60 minutes at 4°C and resuspended in PBS. Microvesicle protein concentrations were measured at 280 nm by using NanoDrop spectrophotometers (NanoDrop 1000; Thermo Scientific, Uppsala, Sweden). Protein quantity of microvesicles was the same in all samples analyzed.

MC activation

LAD2 MCs or human cord blood MCs were activated by means of incubation with 50 μg/mL purified microvesicles from resting T cells (mvT) or microvesicles from activated T cells (mvT*) or 20 μg/mL purified membranes from activated T cells, as previously described.[10] For IgE-mediated activation, cells were sensitized overnight with 500 ng/mL human myeloma IgE (Calbiochem, Merck KGaA, Darmstadt, Germany) and then stimulated with 50 μg/mL anti-human IgE (Dako, Glostrup, Denmark).

RNA extraction and microarray analysis

Total RNA was extracted with the RNeasy Mini Kit (RNeasy Mini Kit; Qiagen, Valencia, Calif) after incubation of LAD2 cells with mvT, mvT*, or membranes derived from activated T cells or by FcεRI cross-linking for 4 hours, according to the manufacturer's protocol.

The microarray experiment protocol is detailed in the Methods section in this article's Online Repository at www.jacionline.org.

Real-time PCR

cDNA was synthesized by using the high-capacity cDNA reverse transcriptase kit (Applied Biosystems, Carlsbad, Calif). Gene expression was determined by using Fast Real-Time PCR with an ABI 7500 Thermal Cycler (Applied Biosystems). Expression of the genes *IL24*, chemokine (C-C motif) ligand 3 (*CCL3*), and chemokine (C-C motif) ligand 7 (*CCL7*) was measured by using specific TaqMan probes (Applied Biosystems; IL-24 [Hs01114270_m1], CCL3 [Hs00234142_m1], and CCL7 [Hs00171147_m1]). Expression of the housekeeping gene β-glucuronidase (*GUSB*) was used for analysis of changes in cycle threshold values.

Flow cytometry

Microvesicles (50 μL) derived from activated T cells (mvT*) were stained with the PKH67 dye, according to the manufacturer's instructions (Sigma-Aldrich). The PKH67-labeled mvT* were incubated with LAD2 cells at 37°C for the indicated time periods. Thereafter, the cells were washed with PBS, labeled with phycoerythrin-CD117 antibody, and analyzed by using flow cytometry (Navios Flow Cytometers, Beckman Coulter) with Kaluza flow cytometry analysis software (Beckman Coulter), as previously described.[10] For inhibition of microvesicle uptake, LAD2 cells were incubated with mvT* at 4°C or were preincubated for 30 minutes with cytochalasin D (Sigma-Aldrich) at a concentration of 20 μmol/L before stimulation for 4 hours at 37°C.

Confocal microscopy

LAD2 MCs were incubated with mvT* labeled with PKH67 for the indicated time periods. At the end of incubation, the cells were washed with PBS, attached to poly-L-lysine–coated slides for 2 hours at 37°C, and fixed with 2% paraformaldehyde. The slides were covered with Gel mount medium (ZYMED Laboratories, Invitrogen Immunodetection, San Francisco, Calif) and analyzed with a Zeiss Laser confocal microscope (Zeiss, Oberkochen, Germany).

Human cytokine assay

Supernatants obtained from the different culture conditions described above were examined for released IL-24 and CCL7 by using a commercial ELISA kit (DuoSet, R&D Systems). CCL3 release was determined by using a commercial ELISA kit (Development kit; PeproTech Asia, Rehovot, Israel).

SDS-PAGE and immunoblotting

Cellular extracts were separated by means of SDS-PAGE with 10% polyacrylamide gels, transferred to nitrocellulose filters, and processed for immunoblotting, as previously described.[10] Immunoreactive bands were visualized with the LAS-3000 imaging system (Fujifilm Corp, Tokyo, Japan).

Immunohistochemistry staining

Skin biopsy specimens were retrieved from the archives of the Meir Medical Center Institute of Pathology after approval of the Institutional Review Board (Helsinki Committee). Skin sections were stained with anti–IL-24 (Abcam, Cambridge, United Kingdom), anti–c-kit, CD117, and anti-MC tryptase (clone AA1; Dako, Glostrup, Denmark) or with Pan Melanoma Cocktail (Biocare Medical, Concord, Calif) for melanocyte staining. Further details are provided in the Methods section in this article's Online repository at www.jacionline.org.