Trans-basement membrane migration of human basophils: role of matrix metalloproteinase-9

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Abstract

In allergic disorders, basophils migrate from the blood stream to inflamed tissue sites. Since trans-basement membrane migration is an important step for local basophil accumulation, we performed a human basophil transmigration assay using a model basement membrane, Matrigel. IL-3 in the upper chamber was critical for basophil trans-basement membrane migration over baseline levels, since none of the chemoattractants placed in the lower chambers induced migration. RANTES, IL-8, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-oxo-ETE) and platelet-activating factor (PAF) significantly up-regulated the transmigration of IL-3-treated basophils. Neutralizing experiments indicated the involvement of β2 integrin and matrix metalloproteinase (MMP)-2/9 in basophil transmigration. Real-time quantitative PCR revealed that basophils constitutively expressed transcripts for MMP-9, and at lower levels, MMP-2, but cell-surface expression was only detected for MMP-9. MMP-9 was also detected in the cytoplasm and culture supernatant of the basophils. Treatment with IL-3 up-regulated the surface level of MMP-9 on the basophils. Our results suggest that basophils possess a unique regulatory mechanism for trans-basement membrane migration which is affected by cytokines, chemoattractants, β2 integrin and MMPs, especially MMP-9. MMP-9 may be critically involved in the pathogenesis of local basophil influx in allergic diseases.

Introduction

Although basophils are the least common cell type among peripheral blood granulocytes, these cells contain potent vasoactive mediators such as histamine in their cytoplasmic granules. When basophils are stimulated by specific antigens or other secretagogues, they are activated and release the granule contents as well as other newly synthesized mediators. Through their release, basophils are thought to be actively involved in the pathogenesis of allergic reactions (1).

Local influx of basophils is a characteristic phenomenon in the late-phase reaction induced by experimental antigen challenge as well as in chronic allergic diseases such as asthma (2–4). We and others have previously reported the underlying mechanisms by which human basophils are recruited from the blood stream to the sites of inflammation. Basophils in the peripheral blood roll and adhere to the vascular endothelium (5), transverse the endothelial cells (6) and move into the interstitium, and finally migrate into the inflamed tissue. Several series of basophil chemoattractants are known, including chemokines (7), cytokines (8), bacterial components (9), complements (10), proteases (9) and specific antigens (11). We recently reported that transendothelial migration (TEM) of basophils through IL-1β-pre-treated human umbilical vein endothelial cells in vitro is regulated by both certain chemokines and cytokines (6).
Trans-basement membrane migration of basophils

In general, the vascular wall is surrounded by an elastic structure named the basement membrane, composed of type IV collagen (CL), laminin (LM), proteoglycans and glycoproteins. Not only tumor cells (12) but also lymphocytes (13, 14), neutrophils (15, 16), mast cells (17) and eosinophils (18, 19) are reported to degrade the basement membrane and move across it by using proteases. Matrix metalloproteinases (MMPs) are a series of proteases that contain zinc ions in their active sites and act as the key proteases by which both tumor cells and inflammatory cells degrade the basement membrane. Furthermore, gelatinases, including MMP-2 and MMP-9, are presumed to be especially important for cell transmigration since these proteases act on type IV CL and other components of the basement membrane. It is reported that in asthmatic airways and other tissues of allergic disorders, MMP-9 expression is highly up-regulated. Thus, local MMP expression is thought to be involved in the pathogenesis of asthma and other inflammatory disorders (20, 21). Recent reports suggest that neutrophils (15) and eosinophils (22) may be important cellular sources of MMP-9 in asthmatic airways, but little is known whether basophils are also a source of MMP-9.

Since there must be a chemical and biological process by which human basophils transverse the basement membrane to enter inflamed tissues, we performed, for the first time, a basophil transmigration assay using an in vitro experimental system, Matrigel, and elucidated the mechanism by which basophils degrade and migrate across the basement membrane.

Methods

Reagents

The following reagents were purchased as indicated: human eotaxin/CCL11 and RANTES/CCL5 (R&D, Minneapolis, MN, USA); MCP-1/CCL2 and IL-8/CXCL8 (PeproTech Inc., Rocky Hill, NJ, USA); Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden); PBS and RPMI 1640 medium (GIBCO, Grand Island, NY, USA); human recombinant CSa and formyl-methionyl-leucyl-phenylalanine (fMLP) (Sigma, St Louis, MO, USA); MMP-2/MMP-9 inhibitor III (Calbiochem, Darmstadt, Germany) (23) and normal swine serum (DAKO, Kyoto, Japan); Human recombinant IL-3 was kindly donated by Kiriin Brewery (Tokyo, Japan). 5-Oxo-6E.8Z,11Z,14Z-eicosatetraenoic acid (5-oxo-ETE), prostaglandin D2 (PGD2) and platelet-activating factor (PAF) C-16 were purchased from Cayman Chemical (Ann Arbor, MI, USA). These lipid mediators were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1.5 mM as stock solutions.

The following antibodies were purchased as indicated: mouse anti-human MMP-2 mAb (IgG1, clone 42-5) (DFC, Toyama, Japan); rabbit polyclonal anti-human MMP-9 antibody (Chemicon International, Temecula, CA, USA); mouse anti-CD18 mAb (IgG1, clone L130) (BD Pharmingen, San Diego, CA, USA); mouse anti-CD29 mAb (IgG1, clone 4B4) (Coulter Immunotech, Marseille, France); mouse anti-CXCR1 mAb (IgG2a, 42705) and mouse anti-CXCR2 mAb (IgG2a, 48311.211) (R&D); control mouse IgG1 (MOPC21) and mouse IgG2a (UPC10) (Sigma); normal rabbit IgG and FITC-conjugated polyclonal swine anti-rabbit Ig (DAKO); FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA) and FITC-conjugated goat anti-human IgE antibody (Biosource International, Camarillo, CA, USA). Mouse anti-CR1 mAb (IgG1, clone 141) and anti-CR3 mAb (IgG1, clone 444) were used for neutralization experiments (6).

Isolation of basophils, eosinophils and neutrophils

Leukocytes were isolated from venous blood obtained from consenting volunteers with no history of atopic diseases. Basophils were semi-purified by density centrifugation using Percoll solutions of different densities (1.080 and 1.070 g ml⁻¹). The purity of these Percoll-separated basophil preparations was 15.8 ± 2.0% (n = 29), and the yield was ~1.1 × 10⁵ basophil ml⁻¹ of peripheral blood. A vast majority of the contaminating cells were lymphocytes, and the preparations contained no eosinophils or neutrophils. For some experiments, the Percoll-separated basophils were further purified by negative selection with MACS beads (Basophil Isolation Kit, Miltenyi BioTech, Belgisch-Gladbach, Germany) according to the manufacturer’s instructions (purity: 98.6 ± 0.4%, n = 25).

Eosinophils were purified by density gradient centrifugation followed by negative selection using anti-CD16-bound beads as previously described (7). After this negative selection, the eosinophil purity was 99.1 ± 0.6% (n = 16).

Human neutrophils were separated by density gradient centrifugation followed by negative selection using anti-CD14-bound micromagnetic beads (Miltenyi BioTech). The purity of neutrophils was 97.7 ± 0.6% (n = 11).

Trans-basement membrane migration assay

A reconstituted basement membrane, Matrigel, was purchased from BD Biosciences Discovery Labware (Bedford, MA, USA). Matrigel is used as an active basement membrane model, and it is composed of 31% CL IV, 56% LM and 8% entactin. Matrigel was rehydrated and pre-incubated in 24-well culture plates (IWAKI, Tokyo, Japan) for 2 h with 1 ml per well of DMEM. Then 500 μl of RPMI medium containing 0.3% HSA and 2 × 10⁴ basophils was added to the upper chamber of the Matrigel, and 750 μl of test reagent in RPMI medium with 0.3% HSA was placed in the lower chamber. After incubation at 37°C in 5% CO₂ for 18 h, unless otherwise noted, cells that had migrated into the lower chambers were collected and stained with 10 μg ml⁻¹ of FITC-conjugated goat anti-human IgE for 30 min at 4°C. The cells were then counted using an Experimental Physics and Industrial Control System, XL System II (Coulter, Miami, FL, USA). Migrated basophils were identified as cells that were strongly positive for IgE, as previously described (6). The total number of basophils was simultaneously analyzed by flow cytometry, using aliquots of cell preparations incubated for 18 h in culture plates. The percent migration was expressed as the percentage of the migrated basophils to the total basophils put in the upper chambers.

Real-time PCR

Real-time quantitative PCR analysis was performed as previously described. In brief, total RNA was extracted from MACS-separated basophils (n = 7), eosinophils (n = 9) or neutrophils (n = 7) from separate donors using RNeasy Mini Kit (Qiagen, Hilden, Germany). For some experiments, MACS-separated basophils were treated with IL-3 at 300 pM in RPMI
with 0.3% HSA for 4 h before RNA extraction. Real-time PCR was performed using the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA). The primers and the probes for MMP-2 and MMP-9 were designed by PE Applied Biosystems. A standard curve was constructed with serial dilutions of specific PCR products, which were obtained by amplifying peripheral leukocyte cDNA as previously described (24).

**Immunohistochemistry**

Cytospins of purified basophils, eosinophils and neutrophils from healthy donors were prepared and fixed with 4% PFA for 20 min at 4°C. After washing three times with Tris-buffered saline (TBS), the slides were treated with ethanol for 1 min. They were then incubated with a blocking solution (TBS containing 2% BSA and 5% normal swine serum) for 2 h at room temperature. Afterwards, the slides were incubated with 10 μg ml⁻¹ of either rabbit anti-MMP-9 polyclonal antibody or control rabbit IgG at 4°C overnight. On the next day, after washing three times with TBS, they were incubated with biotinylated goat anti-rabbit IgG and then treated with alkaline phosphatase-conjugated streptavidin (SLAB-AP Kit, DAKO). The enzyme activity was visualized by staining with New Fuchsin solution (DAKO).

**Flow cytometric analysis of MMP expression**

Surface expression of MMP-2 and MMP-9 was analyzed by flow cytometry using highly purified basophils, eosinophils or neutrophils. Cells were incubated for 30 min at 4°C with 10 μg ml⁻¹ of either anti-MMP-2 or anti-MMP-9 antibodies or control antibody and then stained with FITC-conjugated second antibody at 10 μg ml⁻¹ for 60 min at 4°C. For intracellular staining, highly purified granulocytes were fixed with PBS containing 4% PFA at 4°C for 30 min followed by permeabilization in PBS with 0.1% Tween 20 at 4°C for 30 min. The cells were then stained and analyzed as described above.

**ELISA**

For ELISA assay, 5 × 10⁵ cells ml⁻¹ of purified basophils, eosinophils or neutrophils were cultured in RPMI medium containing 0.3% HSA for 1 h at 37°C, and the supernatant was collected after centrifugation. Immunoreactive MMP-9 was measured using an ELISA kit for MMP-9 (Amersham Biosciences, Buckinghamshire, UK; detection range: 1–32 ng ml⁻¹) by following the manufacturer’s instructions.

**Statistics**

All data are expressed as the mean ± SEM. Differences between values were analyzed by the one-way analysis of variance test. When this test indicated a significant difference, Fisher’s protected least significant difference test was used to compare individual groups.

**Results**

**Trans-basement membrane migration of human basophils**

Basophil trans-basement membrane migration assay was performed using an experimental model, Matrigel, a gel containing basement membrane components, that is, LM, type IV CL, heparan sulfate, proteoglycan and entactin. Various chemokines and lipid mediators were placed in the lower chamber. After 18 h of incubation, ~10–16% of the basophils had transmigrated spontaneously to the lower chamber; however, no apparent induction of transmigration was observed with any of the tested chemokines or lipid mediators (“nil” columns in Fig. 1A). We next included the potent basophil-active cytokine, IL-3, at 300 μM in the upper chamber. As shown in Fig. 1(A), IL-3 in the upper chamber induced statistically significant basophil trans-basement membrane migration even in the absence of chemoattractants in the lower chambers. Moreover, among the chemokines tested, IL-8 and RANTES at 50 nM added to the lower chamber induced a significant increase in migration, compared with the spontaneous migration of IL-3-treated basophils. Other chemokines, including MCP-1 and eotaxin, exhibited weak basophil attracting potency, but it did not reach statistical significance. Complement C5a failed to show significant transmigration-inducing activity. On the other hand, among the lipid mediators tested, 5-oxo-ETE and PAF at 1 μM significantly induced basophil transmigration, but only when IL-3 was included in the upper chamber. Other mediators, such as PGD₂ and fMLP, failed to induce significant migration of basophils.

As shown in Fig. 1(B), both RANTES and 5-oxo-ETE showed concentration-dependent induction of migration of IL-3-treated basophils through Matrigel. In the presence of IL-3 in the upper chamber, maximal migration was induced by RANTES at a concentration of 50 nM and by 5-oxo-ETE at 1 μM, both of which are similar to concentrations capable of inducing basophil migration across bare membranes (25). A kinetic study of basophil migration revealed that basophil trans-basement membrane migration is a slow process which starts after 3 h and gradually continues to increase up to 18 h (Fig. 1C). Although there was wide variation among donors, a small fraction (~10%) of basophils demonstrated spontaneous transmigration across Matrigel at 6 and 18 h in the absence of both IL-3 and chemoattractants (shown as “nil” in Fig. 1C). Importantly, the combination of IL-3 (upper chamber) and RANTES (lower chamber) demonstrated an obvious increase in transmigration at all time points between 3 and 18 h. As shown in Fig. 1(D), both semi-purified and highly purified basophils demonstrated similar transmigration responses to 5-oxo-ETE. Transmigration to RANTES was also readily induced in highly purified basophils (data not shown), suggesting that transmigration of basophils can fully occur in the absence of contaminating leukocytes.

Our present study revealed that RANTES, which binds to CCR1 with higher affinity than CCR3, and IL-8, a ligand of CXCR1 and CXCR2, are more efficient for basophil trans-basement membrane migration than a CCR3 ligand, eotaxin. We thus performed neutralization experiments using blocking antibodies against these chemokine receptors to determine which receptor is responsible for this transmigration. As shown in Fig. 1(E), basophil trans-basement membrane migration toward RANTES was significantly inhibited by both anti-CCR1 mAb and anti-CCR3 mAb. In addition, IL-8-induced transmigration was significantly blocked after treatment of basophils with anti-CXCR1 mAb, but not with anti-CXCR2 mAb. These results clearly show that not only CCR3 but also CCR1...
and CXCR1 on basophils are involved in the transmigration across Matrigel.

Checkerboard analyses of the effect of IL-3 on basophil trans-basement membrane migration revealed that IL-3 in the upper chambers showed dose-dependent induction of basophil transmigration (Fig. 1F). Moreover, this action of IL-3 was non-directional because, when IL-3 300 pM was put in both the upper and lower chambers to eliminate the IL-3 gradient, the migration-inducing effect remained the same.

**Effects of adhesion molecules on basophil trans-basement membrane migration**

Basophils express both β1 (CD49d, CD49e and CD29) and β2 (CD11a, CD11b and CD18) integrins on their surface (5). We performed the migration assay using anti-CD29 antibody and anti-CD18 antibody, which are neutralizing antibodies to β1 and β2 integrins, respectively. Anti-CD18 antibody significantly inhibited both IL-3 plus 5-oxo-ETE-induced trans-basement membrane migration of basophils (Fig. 2). On the other hand, anti-CD29 did not show any apparent inhibitory effect on the migration. The results were similar when we used RANTES at 50 nM as a chemoattractant (data not shown). These results indicate that β2 integrin, but not β1 integrin, plays a central role in trans-basement membrane migration of human basophils.

**Involvement of MMPs in basophil trans-basement membrane migration**

We next examined the involvement of these proteinases in the migration using a recently developed MMP-2- and MMP-9-specific inhibitor that does not inhibit other MMP family members (23). This inhibitor exerted no cytotoxic effect on human basophils, as shown by annexin V and propidium iodide staining (data not shown). When serial dilutions of the reagent were put in the upper chamber together with basophils, trans-basement membrane migration of both semi-purified and highly purified basophil preparations was significantly diminished by up to 75% (Fig. 3). Importantly, both IL-3-induced migration and IL-3 plus 5-oxo-ETE-induced migration were suppressed by this inhibitor. Thus, in parallel with other cell types, MMP-2 and/or MMP-9 are essential for full induction of trans-basement membrane migration of basophils.

Real-time PCR of MMPs

In the next series of experiments, we performed real-time PCR of MMPs using cDNAs from basophils, eosinophils and neutrophils. As shown in Fig. 4(A), the expression levels of MMP-2 were very low in the majority of donors for all three subsets of granulocytes. On the other hand, as shown in Fig. 4(B), MMP-9 transcripts were detected in the granulocyte subsets from all the donors tested. Neutrophils expressed a high level of MMP-9 mRNA, consistent with previous reports (26). We found that the amount of MMP-9 transcripts in basophils was much lower than in neutrophils, but similar to or even higher than in eosinophils. We next tested the mRNA expression levels of MMP-2 and MMP-9 in basophils treated with or without IL-3 at 300 pM for 4 h, but no obvious change was induced by IL-3 (n = 3, data not shown).

**Fig. 1.** Trans-basement membrane migration of human basophils. (A) Percoll-separated basophils were suspended in medium with (filled columns) or without (open columns) IL-3 at 300 pM and subjected to the basophil trans-Matrigel migration assay. Various chemokines or lipid mediators were placed in the lower chamber. After incubation for 18 h, cells that had migrated to the lower chambers were counted. **(B)** Trans-basement membrane migration revealed that IL-3 in the upper chambers showed dose-dependent induction of basophil transmigration (Fig. 1F). Moreover, this action of IL-3 was non-directional because, when IL-3 300 pM was put in both the upper and lower chambers to eliminate the IL-3 gradient, the migration-inducing effect remained the same.

**Fig. 2.** Involvement of adhesion molecules in basophil trans-basement membrane migration. Neutralizing antibodies against CD18 and CD29 or control mouse IgG1 were added at 10 μg ml⁻¹ to the Percoll-separated basophil preparations with IL-3 at 300 pM. Bars represent the SEM (n = 3, data not shown).

**Fig. 3.** Time-course of trans-basement membrane migration of basophils. IL-3 was added to the upper chamber and RANTES was included in the lower chamber. The actual percentages of migrated basophils are shown. The data are representatives of two experiments using basophils from two different donors. **(B)** Both Percoll-separated and MACS-separated basophils were tested for trans-Matrigel migration. IL-3 was added to the upper chambers. Either the vehicle (DMSO) or 5-oxo-ETE at 1 μM was put in the lower chamber. Data are the actual percentages of migrated cells. Bars represent the SEM (n = 4). **P < 0.05 and ***P < 0.01 versus migration of basophils in the absence of IL-3. **(C)** Time-course of trans-basement membrane migration of basophils. IL-3 was added to the upper chamber, and RANTES was included in the lower chamber. The actual percentages of migrated basophils are shown. The data are representatives of two experiments using basophils from two different donors. **(B)** Both Percoll-separated and MACS-separated basophils were tested for trans-Matrigel migration. IL-3 was added to the upper chambers. Either the vehicle (DMSO) or 5-oxo-ETE at 1 μM was put in the lower chamber. Data are the actual percentages of migrated cells. Bars represent the SEM (n = 4). **P < 0.05 and ***P < 0.01 versus migration of basophils in the absence of IL-3.
phils. As shown in Fig. 6, the cytoplasm of neutrophils and basophils showed intense staining for MMP-9, although the control antibody was unable to stain them. However, eosinophils showed almost no staining for MMP-9.

MMP-9 is released from basophils

Finally, we performed MMP-9 ELISA assay using cultured cell supernatants. As previously reported, neutrophils constitutively released MMP-9 abundantly in the medium (38.5 ± 1.2 ng ml⁻¹, n = 3). The culture supernatants of basophils and eosinophils also showed detectable levels of MMP-9 (1.3 ± 0.1 ng ml⁻¹ and 1.4 ± 0.4 ng ml⁻¹, respectively; n = 3); however, the levels were much lower than those of neutrophils. IL-3 at 300 pM was unable to enhance MMP-9 release from basophils (1.3 ± 0.1 ng ml⁻¹, n = 3).

Discussion

Accumulation of basophils in the local tissues is a hallmark aspect in allergic diseases and in late-phase allergic reactions following antigen challenge (2, 3, 28). Analysis of chemical mediators secreted during late-phase reactions has implied the participation of locally migrated basophils in the pathogenesis of allergic inflammation (2, 3). Transmigration across the endothelium and basement membrane is an essential process for recruitment of peripheral blood leukocytes into local tissues, but, for basophils, our knowledge on this step is limited. TEM of basophils has very recently been reported (6), but transmigration of basophils through the basement membrane has not been studied to date. We thus investigated the trans-basement membrane migration of human basophils using Matrigel.

In our preliminary experiments using basophils and eosinophils, we found that higher percentages of basophils migrate through Matrigel compared with eosinophils. That is, >10% and 30% of basophils migrated through Matrigel after incubation for 18 h without any agent in the medium and under enhanced conditions with IL-3 plus RANTES (Fig. 1), respectively. Importantly, trans-basement membrane migration of basophils was significantly enhanced by a basophil-active cytokine, IL-3, in a chemokinetic fashion, and other chemoattractants further enhanced this transmigration in a dose-dependent manner when IL-3 was included in the upper chamber. An indirect effect of contaminating cells on basophil transmigration was unlikely, because essentially the same percentages of transmigration were observed for partially purified and highly purified basophil preparations.

One of the most important characteristics of basophil trans-basement membrane migration is that IL-3 is essential for chemoattractant-induced transmigration. We found that, in the absence of IL-3, none of the tested chemokines or lipid mediators up-regulated basophil transmigration above the control level. On the other hand, in the presence of IL-3, transmigration of basophils was significantly induced by chemokines and lipid mediators in a directional manner. It is widely known that a hematopoietic cytokine, IL-3, is pleiotropic in action. That is, this cytokine acts on mature basophils to potentiate various aspects of cellular functions such as degranulation (29), survival (29), up-regulation of surface...
activation marker expression (30), adherence to endothelium (5) and TEM (6). This study shows that trans-basement membrane migration of basophils is also critically regulated by IL-3. In addition, the results of our present study reveal a unique profile of chemoattractants in basophil trans-basement membrane migration compared with simple chemotaxis or TEM of basophils. Although a selective CCR3 ligand, eotaxin, failed to show significant up-regulation of basophil transmigration, RANTES and IL-8 unexpectedly showed potent induction of trans-Matrigel migration of basophils. The results of neutralizing experiments revealed that CCR1, CCR3 and CXCR1 are involved in the trans-Matrigel migration. The participation of multiple chemokine receptors on basophils in trans-basement membrane migration is in clear contrast to the previous findings that CCR3 plays a central role in basophil TEM and chemotaxis (6, 25), suggesting that different sets of chemokines

Fig. 5. Flow cytometric analysis of MMP-2 (A and B) and MMP-9 (C–I). Highly purified preparations of basophils (A–D), eosinophils (E and F) and neutrophils (G and H) were stained using control antibody (open area) or anti-MMP-2 or -9 antibody (shaded area). Non-fixed (A, C, E and G) and fixed cells (B, D, F and H) were simultaneously stained and analyzed by flow cytometry. (I) Surface MMP-9 was analyzed using basophils cultured for 24 h with or without IL-3 at 300 pM. All data are representatives of three separate experiments using cells from different donors, showing similar results.

Fig. 6. Immunohistochemistry for MMP-9 in basophils, eosinophils and neutrophils. Cytospin preparations of highly purified cells were incubated with either control IgG (upper panels) or rabbit anti-MMP-9 antibody (lower panels) at 10 \mu g ml^{-1}. Representatives of four separate experiments are shown. Original magnifications \times 600.
In addition, MMP-9 is presumed to be involved in airway increased levels of MMP-9 in asthmatic airways (21, 35, 36). In diseases (21, 35). Recent reports have demonstrated instasis (34) but also inflammation associated with allergic various biological situations, including not only tumor metasta-

The molecular mechanism of basophil trans-basement membrane migration is of importance. Adhesion molecules, especially β2 integrin, are important for basophil trans-basement membrane migration. Since β2 integrin is up-regulated by cytokines, including IL-3 (5), and is reported to be involved in other migratory processes such as TEM for basophils (6) and eosinophils (32), this integrin appears to be one of the critical molecules in the pathogenesis of allergic inflammation. Another molecule related to Matrigel trans-migration of basophils appears to be MMPs, which degrade basement membrane components. In parallel with the previous findings that eosinophils utilize proteinases for trans-migration (18), our inhibition experiments found that basophils also use proteinase activity, that is, MMP-2 and/or MMP-9. The results of quantitative real-time PCR and immunological studies collectively suggest that MMP-9 is synthesized, stored in the cytoplasm, expressed on the cell surface and released by basophils. On the other hand, MMP-2 protein expression was very little, if any, in the cytoplasm and on the surface of basophils. Since MMP-9 can degrade matrix 25 times more efficiently than MMP-2, most of the gelatinase activity in basophils may derive from MMP-9. Cell-surface MMP-9 and MMP-9 that has been released will cooperatively help basophils cross the basement membrane. Our flow cytometric analysis revealed that the surface MMP-9 level on basophils is up-regulated by IL-3; this action of IL-3 may, at least partly, account for the enhancement of basophil trans-Matrigel migration by IL-3. Modulation of cell-surface MMP-9 may be especially important since surface-bound MMP-9 is more potent than free MMP-9 in cleavage of the extracellular matrix and is substantially resistant to inhibition by endogenous tissue inhibitors of metalloproteinases (TIMPs) (27). Our preliminary data support this notion: exogenously added TIMPs showed no inhibitory effects on basophil transmigration across Matrigel. Our results clearly show that in addition to neutrophils and eosinophils, basophils comprise a potential source of MMP-9. Our findings differ from the report of Hayashi et al. (33) that MMP-9 was not detected in basophil preparations (33). The reason for the discrepancy between the two studies is unclear, but differences in the sensitivity of the immunohistochemical procedures may be involved. We further tested whether basophil functions are modulated by MMP-9. However, MMP-9 failed to directly activate basophils since exogenous MMP-9 did not induce intracellular Ca influx or up-regulate the expression of the adhesion molecule, CD11b, on the surface of human basophils (data not shown).

It has been postulated that MMP-9 may be involved in various biological situations, including not only tumor metasta-

of the action of MMP-9 (presumably in its soluble form) by endogenous TIMPs may also occur (37). Recent studies by others and our present study collectively suggest that locally increased levels of MMP-9 in allergic disorders may cause basophils to accumulate in the inflammatory sites and that the accumulated basophils participate in exacerbation of allergic inflammation in cooperation with other types of inflammatory cells. Thus, it will be of special interest to elucidate the extent to which manipulation of local MMP-9 activity can alter leukocyte influx in allergen-induced airway inflammation, presumably via inhibition of trans-basement membrane migration.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CL</td>
<td>collagen</td>
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<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<td>FMLP</td>
<td>formyl-methionyl-leucyl-phenyalanine</td>
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<tr>
<td>LM</td>
<td>laminin</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
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<tr>
<td>5-oxo-ETE</td>
<td>5-oxo-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid</td>
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<tr>
<td>PAF</td>
<td>platelet-activating factor</td>
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<tr>
<td>PGD2</td>
<td>prostaglandin D2</td>
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<tr>
<td>TBS</td>
<td>Tris-buffered saline</td>
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<tr>
<td>TEM</td>
<td>transendothelial migration</td>
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<td>TIMP</td>
<td>tissue inhibitor of matrix metalloproteinase</td>
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