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Augmentation of RANTES-Induced Extracellular Signal-Regulated Kinase Mediated Signaling and T Cell Adhesion by Elastase-Treated Fibronectin

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T cells migrating across extracellular matrix (ECM) barriers toward their target, the inflammatory site, should respond to chemoattractant cytokines and to the degradation of ECM by specific enzymes. In this study, we examined the effects of RANTES and ECM proteins treated with human leukocyte elastase on T cell activation and adhesion to the ECM. We found that human peripheral blood T cells briefly suspended with RANTES (0.1–100 ng/ml) had increased phosphorylation of their intracellular extracellular signal-regulated kinase (ERK), a mitogen-activated protein kinase involved in the activation of several intracellular downstream effector molecules implicated in cell adhesion and migration. Consequently, a small portion (12–20%) of the responding cells adhered to fibronectin (FN). However, when the T cells were exposed to RANTES in the presence of native immobilized FN, laminin, or collagen type I, ERK phosphorylation was partially inhibited, suggesting that this form of the ECM proteins can down-regulate RANTES-induced intracellular signaling. In contrast, when the T cells were exposed to RANTES in the presence of elastase-treated immobilized FN, but not to elastase-treated laminin, ERK phosphorylation was markedly increased. Furthermore, a large percentage (30%) of RANTES-activated T cells adhered to the enzymatically treated FN in a β1 integrin-dependent fashion. Thus, while migrating along chemotactic gradients within the ECM, T cells can adapt their adhesive performance according to the level of cleavage induced by enzymes to the matrix. The Journal of Immunology, 2001, 166: 7121–7127.

The translocation of leukocytes, including T lymphocytes, from the vascular system into the extracellular matrix (ECM), which functions as a scaffold for cell adhesion and tissue architecture, is a central event associated with inflammation and tissue injury. Moving through this condensed meshwork of macromolecules, the migrating leukocytes can dynamically affect the ECM by secreting proinflammatory molecules (i.e., cytokines and enzymes), and thus can receive a newly formed set of contextual signals. The resulting changes in the ECM composition most likely play a role in transducing regulatory signals into the moving cells, and thereby coordinate their behavior (1–3). Such putative activities of modified ECM constituents further emphasize the importance of matrix-degrading enzymes during inflammation, tissue destruction, and tumor growth (4–6). T cell movement within the ECM toward inflamed areas is governed by chemical gradients formed by soluble or ECM-complexed proinflammatory chemoattractants (e.g., chemokines) (7–9). One such chemokine is RANTES, which was shown to be instrumental in the pathogenesis of inflammation in vivo (10, 11), the induction of β1 integrin-mediated T cell adhesion to ECM (12), and the elicitation of the directional migration of lymphocytes in ECM-like gels in vitro (8). RANTES activates monocytes and T cells by binding to several G-coupled receptors, and thereby induces rapid cell polarization and chemotaxis. These processes are associated with phosphatidylinositol 3 kinase signaling (13), a transient increase in cytosolic Ca2+ concentration, and tyrosine phosphorylation of several proteins, such as FAK, ZAP-70, and paxillin. The latter molecules form a set of cytoskeletal-based elements implicated in the formation of focal adhesion contacts, cell adhesion, and activation (14). Recently, it was shown that stromal cell-derived factor (15), a potent T cell chemoattractant (8, 15), induces activation via another family of signaling moieties, the mitogen-activated protein (MAP) kinases (16). Activation of the MAP kinase, designated extracellular signal-regulated kinase (ERK), includes a cascade of events involving Ras, Raf, and MAP/ERK (MEK) kinase (17). Interestingly, ERK has also been shown to mediate the adhesion of EL4 cells by phosphorylating paxillin (18) and to induce the phosphorylation and activation of the myosin light chain kinase. Both of these processes are required for cell migration (19). However, to date, the involvement of ERK in RANTES-associated adhesion of T cells to ECM has not been reported.

In this study, we have assumed that the degradation of cell-adhesive ECM components, such as fibronectin (FN), evokes cellular responses involved in the ongoing inflammatory response. Elastase, which is secreted from various types of cells, including leukocytes, can degrade ECM proteins by virtue of its wide range of protein substrate specificities (20–22). We investigated whether immobilized FN, either intact or elastase degraded, can affect T
cell activation and intracellular phosphorylation of ERK in response to RANTES. Our findings indicate that RANTES stimulation of T cells, conducted on immobilized intact FN, results in a marked inhibition of ERK phosphorylation, compared with T cells in suspension. In contrast, when FN was pretreated with elastase, ERK phosphorylation in RANTES-treated T cells and T cell adhesion to the modified ECM glycoprotein were markedly enhanced. Hence, we propose that the chemical integrity of the ECM may contribute to the regulation of lymphocyte functions.

Materials and Methods

Reagents

The following reagents and chemicals were used: collagen type I (CO-I) and laminin (LN; Collagen; ICN Pharmaceutica!s, Costa Mesa, CA); FN (Chemicon, Temecula, CA); BSA, phosphatase inhibitor cocktail, the Antibody (R&D) and -Leu-Asp-Al (LDV)-containing peptides, and PMA (Sigma, St. Louis, MO); HEPES buffer, antibiotics, heat-inactivated FCS, sodium pyruvate, and RPMI 1640 (Kibbutz Be!-Haemek, Israel); human rIL-2 (sp. act., 18 10^6 U/mg; Chiron, Amsterdam, The Netherlands); and human rRANTES (PeproTech, Rocky Hill, NJ). Tissue culture plates were purchased from Becton Dickinson Labware (Franklin Lakes, NJ), mAb directed against human β1 integrin subunits α5, α6, and α8 were obtained from Serotec (Oxford, U.K.), and anti-phosphorylated ERK (pERK) (clone B180) mAb was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and from Serotec (Oxford, U.K.), according to the manufacturer's instructions. The human neutrophil elastase was purchased from ICN Biomedica!s (Costa Mesa, CA), and Na_2^{31}CrO_4 was purchased from Amersham Pharmacia Biotech, Little Chalfont, U.K.). The ERK-inhibiting compound, designated PD098059, as well as the rabbit anti-human Ab against ERK were supplied by R. Zeger (The Weizmann Institute of Science, Rehovot, Israel).

T cells

Purification of human T cells was conducted as previously described (12, 23). Briefly, T cells from the peripheral blood of healthy human donors were isolated on a Ficoll gradient, washed, resuspended in PBS containing 5% heat-inactivated FCS, and incubated (45 min, 37°C) in a 7% CO_2-humidified atmosphere on prepacked nylon wool columns (Fenwal Laboratories, Deerfield, IL), according to the manufacturer's instructions. The nonadherent cells were eluted and washed, and the remaining platelets were removed by centrifugation (700 rpm, 15 min). To remove monocytes, the cells were incubated (2 × 45 min, 37°C) in tissue culture-grade flasks. The nonadherent cells were collected, washed, counted, and cultured (3 × 10^5 cells/ml; 7% CO_2-humidified atmosphere) in RPMI 1640 containing 10% heat-inactivated FCS, supplemented with rIL-2 (200 U/ml) for 7 days. The resulting PBL culture thus contained ≥94% CD^3^ T cells.

T cell adhesion assay

T cell adhesion to immobilized FN was done as previously described (8, 12, 20). Briefly, flat-bottom microtiter well plates (Becton Dickinson Labware) were precoated with FN (1 μg/well, 1 h, 37°C) and washed, and the remaining binding sites were blocked with 0.1% BSA. Next, 1^{3}Cr-labeled T cells (2 × 10^5 cells in 100 μl of RPMI containing 0.1% BSA) were added to the wells. Where indicated, PMA (50 ng/ml) or RANTES (1–100 ng/ml) was added to the wells together with the cells. The plates were centrifuged (800 rpm, 2 min), further incubated (60 min, 37°C, 7% CO_2-humidified atmosphere), and then washed. The adherent cells were counted, and cultured (3 × 10^5 cells/ml) in RPMI 1640 containing 10% heat-inactivated FCS, supplemented with rIL-2 (200 U/ml) for 7 days. The resulting PBL culture thus contained ≥94% CD^3^ T cells.

Coating culture plates with ECM glycoproteins and treatment of coated proteins

Flat-bottom tissue culture plates (24 wells) were coated (1 h, 37°C) with 25 μg/ml FN, LN (1.25 μg/ml), or CO-I (12.5 μg/ml). After incubation, the coated wells were washed with warm PBS, and where indicated, treated with human neutrophil elastase (50 ng/ml, 18 h, 37°C), which was discarded before adding the T cells. Similar tissue culture wells, which were coated with untreated (native) FN, were preserved in PBS under the same conditions. Mechanical disruption of FN was performed with a syringe needle (25G; eight scratches on the diameter of the well).

Western blotting analysis

After exposing the T cells to the various treatments and incubation of the intact or enzymatically treated ECM proteins, T cells were lysed (30 min, 4°C) in 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 1:1000 phosphatase inhibitor mixture. Next, the lysates were centrifuged, and the solubilized proteins were separated by SDS-PAGE; they were then transferred to a cellulose membrane (Schleicher & Schuell, Dassel, Germany) and subjected to 90 V for 1 h. After blocking the membranes with 5% milk (2 h, 22°C) in TBST (20 mM Tris (pH 7.5), 135 mM NaCl, 0.1% Tween 20), the membranes were incubated (18 h, 4°C) with mAb anti-pERK (diluted 1/1000). pERK was detected using a HRP-conjugated secondary Ab (1 h, 20°C), followed by visualization using an ECL system. Next, we verified that equal amounts of proteins were tested in each sample. This was performed by stripping the membranes and reexposing them to Western blotting using an Ab directed against the total form of ERK (eERK). All the bands were analyzed densitometrically using the NIH Image Program. In each figure, the level of phosphorylation, in percent, was calculated as follows: (the OD of a given protein band of pERK/the corresponding OD of total ERK) × 100 (i.e., pERK:ERK). The amount of pERK in the treated T cells was calculated according to the level of ERK phosphorylation in untreated cells (in the absence of adhesive substrates), which was considered as 100%.

Statistical analysis

The data obtained were statistically calculated using Student's t test. The difference between the groups was considered statistically significant at p < 0.05.

Results

RANTES induces the phosphorylation of ERK in human T cells in suspension

It was previously shown that RANTES induces T cell adhesion to ECM components and the chemotactic migration of T cells by binding to specific G protein-coupled receptors (14, 24). T cell adhesion and migration require intracellular activation processes that are not yet fully delineated. These cytoskeletal-associated modifications can affect the migratory phenotype of the responding cells. One such molecular candidate for intracellular activation is ERK (17, 25). Therefore, we examined the putative phosphorylation of ERK in T cells treated with RANTES. Human T cells were purified from the peripheral blood of healthy donors and maintained under tissue culture conditions in medium containing IL-2 for 7 days. The T cells were then washed and subjected to a 10-min exposure to different concentrations of human rRANTES, and then lysed. The amount of tyrosine phosphorylation of ERK in control and treated T cells was analyzed by Western blotting. The results indicated that RANTES induced the activation of ERK phosphorylation (pERK) in a dose-dependent manner. As shown in Fig. 1, this induction, which was evident after exposing the cells to RANTES concentrations as low as 0.1 ng/ml, reached its plateau of response at 10–100 ng/ml (p < 0.05, comparing the densitometric units of pERK in treated vs untreated T cells). Note that the minimal effective dose was 0.1 ng/ml; lower concentrations of RANTES failed to induce pERK. ERK phosphorylation, which began after a 2-min exposure to RANTES, reached its maximal value after 10 min, and declined to background levels after 20 min of incubation (Fig. 1). Thus, a short exposure of human T cells to adhesion/migration-inducing amounts of RANTES results in the intracellular phosphorylation of ERK.

RANTES-induced pERK plays a role in T cell adhesion to FN

Next, we investigated whether the RANTES-induced activation of ERK in T cells is linked to the ability of the chemokine to induce T cell adhesion to FN. For this purpose, human T cells were radioactively labeled with ^{51}Cr and added to microtiter wells that were precoated with FN; where indicated, RANTES (1–100 ng/ml) was added to the tissue cultures. To further analyze the involvement of ERK signaling in T cell-FN interaction, we first incubated some T cells with PD098059 (25 μM), a selective inhibitor of
activated ERK may be involved in the adhesion of only a minority of the unstimulated human T cells. The results also indicate that RANTES significantly induced, in a dose-dependent manner, the adhesion of resting T cells to FN (17–24% at 1–100 ng/ml). Note that although RANTES apparently induced pERK already at 0.1 ng/ml (Fig. 1), the minimal dose of RANTES required for adhesion was 1 ng/ml (Fig. 2). This suggests that intracellular mechanisms other than ERK activation are required for optimal RANTES-induced T cell adhesion to FN.

If the T cells were pretreated with PD908059, a significant inhibition (i.e., 35–50%; *p < 0.05*) of T cell adhesion occurred. Although several activation pathways may be involved in RANTES-induced T cell adhesion to FN, our results, indicating that PD908059 inhibits chemokine-induced T cell adhesion, further support the notion that ERK activation is involved in the proadhesive effect of RANTES. Thus, the chemoattractant and proadhesive chemokine RANTES induces the phosphorylation of ERK, and this intracellular event in turn contributes to the FN-binding capacities of responding lymphocytes.

Native, immobilized FN, LN, and CO-I inhibit RANTES-induced pERK in T cells

It is likely that T cells can rapidly sense their microenvironment and respond immediately by virtue of several versatile families of receptors that specifically bind different groups of molecules in the inflammatory context. We assumed that the concurrent exposure of T cells to immobilized ECM glycoproteins, such as CO-I, LN, and FN, as well as to RANTES, should affect the amount of phosphorylation of their intracellular ERK. Therefore, T cells were exposed to RANTES (100 ng/ml; 2 and 10 min) while being incubated on the immobilized ECM substrates. Next, the cells were lysed and the amount of pERK was determined by Western blotting. The results, shown in Fig. 3, indicate that the ECM proteins inhibited the phosphorylation of ERK in the ECM glycoprotein-interacting T cells to the background level, even in the absence of RANTES. However, in the presence of RANTES, as little as a 2-min exposure of the T cells to the chemokine was enough to induce a pronounced (*p < 0.05*) activation of ERK phosphorylation. Note that the level of this pERK was even higher when the incubation time

MEK, and consequently of ERK. In preliminary dose-response assays, using Western blotting techniques, we found that the optimal ERK-inhibiting concentration of PD098059 in RANTES-activated T cells was 25 μM (data not shown). T cell adhesion, determined by the amount of radioactivity associated with the immobilized FN substrate, was measured after 60 min. The results, shown in Fig. 2, indicate that PD098059 only slightly decreased the background level of T cell adhesion to FN. This indicates that

released human T cells (5 × 10⁶ in 250 μl of RPMI with 0.1% BSA per sample) were incubated with RANTES (37°C, 10 min). The T cells were then lysed in a buffer containing phosphatase inhibitors. The level of ERK phosphorylation was analyzed by Western blotting using specific mAb against pERK. Analysis of the total loaded proteins was conducted by reexposing the membranes to Western blotting using Ab anti-ERK (tERK). The SDS-PAGE profile represents one experiment of three. Densitometric analysis of pERK is shown. The level of phosphorylation was calculated as follows: (the OD of a given protein band of pERK/the corresponding OD of tERK) × 100. The amount of ERK phosphorylation in the treated T cells was calculated according to the level of pERK in untreated cells (in the absence of adhesive substrates), which was considered as 100%. The densitometric histograms ± SD were calculated from the results of three different experiments.

FIGURE 2. RANTES-induced T cell adhesion to FN involves ERK activation. Human T cells were labeled with ³¹Cr, and then some cells were treated (30 min, 37°C) with the ERK-inhibiting compound PD098059 (25 μg/ml). The T cells were then seeded on a FN-coated microtiter well plate together with RANTES (100 ng/ml). After incubation, the nonadherent T cells were removed, the adherent cells were lysed, and the percentage of T cells that had adhered was determined. Nonstimulated T cells or cells treated with only PD098059 served as a control. The results represent the mean ± SD of four experiments.

FIGURE 3. Immobilized FN, LN, and CO type I down-regulate RANTES-induced phosphorylation of ERK in T cells. T cells were seeded on a 24-well tissue culture plate (5 × 10⁶ cells/well) that was precoated with FN (6.5 μg/well), LN (1.25 μg/well), or CO-I (12.5 μg/well). RANTES (100 ng/ml) was then added to the tissue cultures. After 2- and 10-min incubations at 37°C, the cells were lysed and ERK phosphorylation was determined by Western blotting. The SDS-PAGE shown is one experiment representative of five. Quantitative analysis of the average amount of ERK phosphorylation in the five experiments was conducted as described in Fig. 1.
SDF-1, ERK in T cells, indicated that the matrix proteins did not affect the apparent inhibitory effect of native FN on RANTES-activated T cells, by blocking the FN-specific VLA-4 and VLA-5 receptors. Control T cells were preincubated with blocking mAb (5 μg/ml), which also activates ERK in T cells, indicated that the matrix proteins did not affect the SDF-1α-induced ERK phosphorylation (data not shown). Hence, FN, LN, and CO-I can inhibit RANTES-induced phosphorylation of ERK in T cells, suggesting that these ECM ligands, when presented to T cells in their native immobilized forms, deliver important regulatory signals to RANTES-triggered T cells.

Next, we examined the role of matrix-specific β1 integrins in the apparent inhibitory effect of native FN on RANTES-activated T cells, by blocking the FN-specific VLA-4 and VLA-5 receptors (αvβ1 and αvβ5, integrins, respectively). Briefly, T cells were pretreated with blocking anti-VLA-4 and anti-VLA-5 mAb, as well as with soluble FN, and the prototypic FN-derived cell-adhesive peptides, LDV and RGD, which block the recognition sites of VLA-4 and VLA-5, respectively. Control T cells were preincubated with anti-VLA-2 mAb. First, we confirmed that these blocking substances had no activating effect of their own on ERK phosphorylation (Fig. 4). Next, the amount of phosphorylation of ERK was determined after short incubations of T cells on immobilized FN with or without RANTES (100 ng/ml). Similar to the findings summarized in Fig. 3, the results indicated that the RANTES-induced pERK in T cells was inhibited by immobilized FN (Fig. 4). However, blocking of the FN-specific integrins by soluble FN (100 μg/ml), as well as anti-VLA-4 and anti-VLA-5 mAb, but not anti-VLA-2 mAb, markedly abolished the pERK-inhibiting effect of FN. Moreover, blocking the FN-specific integrins with RGD and LDV peptides also resulted in the abrogation of the inhibiting effect of FN. Thus, the FN-mediated inhibition of RANTES-induced activation of ERK in T cells is mediated through the recognition of the ECM glycoprotein by its specific integrins.

**Elastase-treated FN enhances the amount of RANTES-induced pERK in responding T cells**

We assumed that T cell behavior in an inflamed area is influenced by the combinations of signals that these cells perceive from their

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**FIGURE 4.** Inhibition of RANTES-induced ERK phosphorylation by intact immobilized FN is a β1 integrin-dependent process. T cells were preincubated (30 min, 4°C) with blocking mAb (5 μg/protein per 10⁶ cells) against VLA-4 (αvβ1) and VLA-5 (αvβ5), as well as with LDV and RGD peptides (500 and 100 mg/ml, respectively). The cells were then washed and placed onto a plastic surface precoated or not precoated with FN. RANTES was added where indicated. After a 10-min incubation at 37°C, the T cells were lysed and analyzed by Western blotting with specific anti-pERK mAb. Anti-VLA-2 mAb was used as a control. The SDS-PAGE shown is one experiment representative of three. Quantitative analysis of the average amount of ERK phosphorylation in the three experiments was conducted as described in Fig. 1. T cells pretreated with the inhibitory substances, in the absence of RANTES, served as a control.

**FIGURE 5.** Disruption of ECM proteins by elastase results in the loss of the ability of FN, but not LN, to decrease RANTES-induced ERK phosphorylation. Tissue culture wells (of a 24-well plate) were precoated with FN or LN. The unbound proteins were washed, and the immobilized ECM glycoproteins were disrupted mechanically (mech) with a fine syringe needle. Then, the solutions were discarded, and the T cells were added (5 × 10⁶ cells/well). Next, RANTES (100 ng/ml) was added to the indicated wells, the plate was incubated (10 min, 7% CO₂, at 37°C) in humidified atmosphere, the cells were lysed, and the level of ERK phosphorylation in the cell lysates was determined by Western blotting. The SDS-PAGE shown is one experiment representative of five. Quantitative analysis of the amount of ERK phosphorylation, of one experiment representative of five, was conducted as described in Fig. 1.
context; these signals can be in the form of cytokines, and are influenced by the physical state of the ECM, which can be affected by ECM-degrading enzymes (1). Elastase is a major ECM-degrading enzyme released by neutrophils and T lymphocytes. To examine the nature of signals transmitted to passing T cells by an inflamed ECM environment, we chose to analyze the effects on pERK in T cells within an environment containing both elastase-treated FN and RANTES.

First, we examined the amount of pERK in FN-interacting human T cells in the absence of RANTES. The results, shown in Fig. 5, show that elastase-treated FN loses its ability to inhibit the basal level of ERK activation in untreated T cells. Moreover, such modified FN enhances the activation of ERK to some degree. Next, we examined the amount of pERK in the lysates of T cells that were exposed to RANTES and incubated for 10 min on FN or LN (Fig. 5, A and B, respectively). The ECM glycoproteins were either left intact or pretreated mechanically or proteolytically by disruption with a 25-gauge syringe needle or by elastase (50 ng/ml, 18 h), respectively. The treated glycoproteins were washed before adding the T cells. The results, shown in Fig. 5A, indicate that the amount of activated ERK was increased by exposing the cells to RANTES, irrespective of whether the T cells were cultured on intact or elastase-disrupted FN, although the amount of pERK in T cells in the presence of elastase-treated FN was significantly higher. Note that this enhancing effect of disrupted FN on pERK was not the result of nonspecific damage; mechanical impairment of FN does not change the level of pERK in T cells, as compared with intact FN (Fig. 5A).

Interestingly, pretreatment of immobilized LN with elastase or by mechanically disrupting the glycoprotein did not result in any apparent modification in the levels of ERK phosphorylation (Fig. 5B). Therefore, in contrast to the effects of native ECM proteins, elastase treatment of FN appears to specifically endow the glycoprotein with stimulatory properties that are evident in the enhancement of pERK in resting or RANTES-treated T cells.

**Treatment of FN with elastase results in increased RANTES-induced T cell adhesion**

The previous experiment indicated that elastase-treated FN further stimulates the RANTES-activated T cells by increasing ERK phosphorylation. Therefore, we investigated whether this phenomenon also influences T cell adhesion to the treated matrix protein. For this purpose, radiolabeled T cells were incubated on FN, intact or pretreated with elastase, in the presence or absence of RANTES. The amount of adhering cells was determined after a short incubation (1 h). The results, shown in Fig. 6, show that although the elastase-treated FN had an activatory effect on ERK phosphorylation (Fig. 5), such FN had no significant proadhesive effect on nonstimulated T cells. In T cells cultured on the intact glycoprotein, RANTES, and to a much greater extent PMA, induced substantial T cell adhesion to FN (14 and 35% adhesion, respectively). In contrast, incubating the T cells on the elastase-treated FN resulted in pronounced up-regulation (26%; p < 0.05) in the adhesion of T cells exposed to RANTES. The ability of RANTES to induce T cell adhesion to the enzymatically treated FN was a β1 integrin-dependent process; mAb anti-VLA-4 and anti-VLA-5 abrogated the adhesion of T cells to the native and the elastase-treated FN (p < 0.05). Thus, FN, disrupted by elastase, shows enhanced proadhesive properties. The control mAb anti-VLA-2, as expected, did not affect the binding of RANTES-activated T cells to the native FN, but exhibited a small inhibitory effect on T cell adhesion to the elastase-treated FN. This suggests that the treated FN may expose new cell adhesion epitopes, which are now recognized by integrins other than VLA-4 and VLA-5.

**Discussion**

The extravasation of immune cells through blood vessel wall barriers and the ECM, a process that occurs in inflammation, is mediated by chemokine and cytokine receptors and requires the secretion of various types of tissue-specific degrading enzymes (1). In this sense, the inflamed ECM context is inherently dynamic, containing different combinations of inflammatory mediators and signals coming from damaged tissue and cells. The present study was undertaken to further understand the regulatory properties of ECM moieties, either enzymatically treated or native, in modifying T cell behavior while responding to RANTES, a classical T cell chemoattractant. We hypothesized that such enzymatic modifications of the ECM composition may affect the role the ECM plays in transducing regulatory signals into moving immune cells in its vicinity to coordinate their behavior (1, 2).

Recent evidence suggests that various enzymes, primarily known for their ECM-specific degrading activities, can also have versatile functions that may influence leukocyte behavior. For example, enzymatically obtained fragments of LN were shown to
have a promigratory effect on human neutrophils (22), and fragments of FN can modify the expression of VLA-5 and affect the migration of monocytes (26). We have shown that heparinase, a heparin sulfate-specific endoglycosidase, can degrade the ECM and release a specific disaccharide that inhibits delayed-type hypersensitivity in mice, and that this effect is accompanied by attenuation of the production of TNF-α by activated T cells (27). In addition, it has been shown that matrix metalloproteinases, metalloelastase, and gelatinase A can generate angiotatin in vivo (28), and degrade the proinflammatory mediators monocyte chemotactic protein-3 (29), SDF-1α (30), and IL-2 (23).

Elastase was chosen because of its context-dependent and versatile substrate-specificity functions in immune cell migration. Elastase is a serine proteinase with a broad spectrum of matrix substrates, such as FN, LN, and CO (19, 20, 31, 32). However, the biological activities of elastase are not restricted to ECM degradation. Elastase may modulate the availability and proadhesiveness of the integrin Mac-1 (CD11b/CD18) by binding to neutrophils and monocytes (33). Cleavage of the T lymphocyte surface molecules CD2, CD4, and CD8 by elastase (34), as well as the proteolytic cleavage of surface ICAM-1 receptors (35) can probably temporarily impair T cell physiology in inflammatory diseases.

Previously, RANTES, a G protein-coupled receptor-specific β chemokine, was shown to activate the homotypic aggregation of T cells, as well as to bind T cells to ECM, in LFA-1/ICAM-3-dependent and β1 integrin-dependent manners, respectively (12, 36). These effects of RANTES were attributed to its ability to induce tyrosine and phosphatidylinositol 3 kinase activities with adhesion-related molecules in T cells (13, 14). In this study, we have demonstrated that RANTES activates, in a dose-dependent fashion, the phosphorylation of tyrosine residues on ERK in human T cells, while being in suspension. The maximal ERK phosphorylation occurred with 10 –100 ng/ml of the chemokine (Fig. 1); such concentrations were also shown to induce T cell adhesion to and migration through the ECM (8, 12). This effect is rapid; it became apparent after only 10 min of exposing the T cells to the activating concentrations of the chemokine. Although this activating effect of RANTES on the phosphorylation of ERK has been demonstrated on IL-5-primed eosinophils (25), no previous studies have examined these effects in T cells. It was previously shown that the phosphorylation of ERK is linked to cell adhesion and migration (19), because its activation was found to lead to downstream signaling events, such as phosphorylation of Ser/Thr residues on paxillin, a docking protein involved in cell adhesion (18, 37). Interestingly, our results also indicated that pERK participates in RANTES-induced T cell adhesion to FN, because blockade of MEK (and as a result of ERK) markedly decreased the RANTES-induced adhesion of T cells to FN (Fig. 2). In addition to these observations, low quantities of RANTES-treated T cells still adhered to FN, indicating that the inhibiting effect of native FN is not an “all or none” phenomenon. In contrast, when FN (but not LN) was pretreated with elastase, there was a marked increase of ERK phosphorylation, as well as cell adhesion.

Under physiological conditions, the ECM is preserved intact. However, during inflammation, the migrating cells, as well as tissue resident cells, secrete matrix-degrading enzymes that can remodel the otherwise quiescent ECM to possess an activatory phenotype. As shown in this study, such an event can be caused by elastase. Treatment of FN (but not LN or CO-I) by elastase not only abrogated the inhibitory capacity of the intact glycoprotein on ERK phosphorylation, but also activated ERK within the interacting lymphocytes. Interestingly, T cell adhesion to elastase-treated FN was also slightly increased (Fig. 6), indicating that ERK is indeed involved in T cell adhesion to elastase-treated FN or its residual molecular fragments. Indeed, in the presence of both RANTES and the enzymatically treated FN, ERK phosphorylation and cell adhesion were markedly enhanced. This suggests that the FN-interacting T cells can appropriately and rapidly adapt their biochemical and cellular behavior to their inflamed context.

The exact chemical nature underlying the modification of native FN by elastase, which transforms the glycoprotein into a T cell-activatory moiety, remains to be determined. We can only speculate that such treatment of FN (20, 21) exposes cryptic sites within the macromolecule that consequently evokes T cell responses. These new epitopes, together with RANTES, can collaborate to transmit enhanced signals into the T cells. It also remains to be investigated whether other ECM-specific enzymes, in addition to elastase, can also convert the signaling capacities of ECM into its stimulatory phenotype. Be that as it may, our results contribute additional proof that the importance of elastase and related enzymes should be viewed as far beyond their mere degradation of tissues during the inflammatory and wound-healing processes. Another question raised by our findings is whether different subsets of human T cells (e.g., CD4 and CD8, memory and naive, Th1 and Th2) respond differently to chemokines in the context of the elastase-treated ECM moieties.

Taken together, these findings represent an example of an autoregulatory mechanism within T cells, in the context of the ECM, while the inflammatory process develops. In an intact ECM milieu, when there is no apparent need for migrating immune cells, the ECM possesses an inhibitory phenotype; therefore, cell adhesion occurs only to a minimal degree. In contrast, when the ECM is enzymatically injured, it signals activatory messages to passing T cells. We postulate that if the constituents of ECM is considered, together with cytokines, as symbols in an intercellular immunological language of past experience (3), then enzyme-treated matrix molecules can be viewed as an additional set of words of the molecular language of inflammation.

References


