Investigation of Chemotactic Activities in Differentiated HL-60 Cells by a Time-lapse Videomicroscopic Assay

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ABSTRACT

Background: Chemotaxis is one of the cardinal functions of leukocytes, which enables them to be recruited efficiently to the right place at the right time. Analyzing chemotactic activities is important not only for the study on leukocyte migration but also for many other applications including development of new drugs interfering with the chemotactic process. However, there are many technical limitations in the conventional in vitro chemotaxis assays. Here we applied a new optical assay to investigate chemotactic activities induced in differentiated HL-60 cells. **Methods:** HL-60 cells were stimulated with 0.8% dimethylformamide (DMF) for 4 days. The cells were analyzed for morphology, flow cytometry as well as chemotactic activities by a time-lapse videomicroscopic assay using a chemotactic microchamber bearing a fibronectin-coated cover slip and an etched silicon chip. **Results:** Videomicroscopic observation of the real cellular motions in a stable concentration gradient of chemokines demonstrated that HL-60 cells showed chemotaxis to inflammatory chemokines (CCL3, CCL5 and CXCL8) and also a homeostatic chemokine (CXCL12) after DFM-induced differentiation to granulocytic cells. The cells moved randomly at a speed of $6.99 \pm 1.24\,\mu$m/min (n=100) in the absence of chemokine. Chemokine stimulation induced directional migration of differentiated HL-60 cells, while they still wandered very much and significantly increased the moving speeds. **Conclusion:** The locomotive patterns of DMF-stimulated HL-60 cells can be analyzed in detail throughout the course of chemotaxis by the use of a time-lapse videomicroscopic assay. DMF-stimulated HL-60 cells may provide a convenient in vitro model for chemotactic studies of neutrophils. **(Immune Network 2006;6(2):76-85)**

Key Words: Chemotaxis, videomicroscopy, HL-60 cells, leukocyte migration, boyden chamber, transwell assay

Introduction

HL-60 is a promyelocytic cell line that can be induced to differentiate into several lineages of myeloid cells, including monocytes, neutrophils and eosinophils (1-3). Dimethylformamide (DMF), dimethylsulfoxide (DMSO), and retinoic acid (RA) can induce HL-60 cells to acquire phenotypic and functional characteristics of granulocytes (4,5), whereas monocytic characteristics can be acquired by stimulation with phorbol myristate acetate (PMA) or vitamin-D (6). HL-60 provides one of the convenient in vitro models for the study of phenotypic as well as functional maturation of myeloid cells (7). Many investigators including us have investigated on the functional maturation of HL-60 cells during in vitro differentiation (4,8), and it has been reported that HL-60 cells acquire chemotactic activities when they are differentiated to granulocytes by inducing with DMSO or all-trans RA (5).

Chemotaxis is one of the cardinal functions of leukocytes as they are intrinsically motile and have to be at the right place at the right time for their proper roles (9,10). In many pathological conditions, the chemotactic activities of leukocytes may be changed and its scientific analysis is critical not only for the understanding of the pathological processes but also for

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the development of appropriate treatment strategies (11). Furthermore, chemotaxis is involved in many other biological processes, such as tumor metastasis, tissue development and regeneration, etc (12,13). Accordingly, it is very important, in many situations, to analyze the in vitro chemotactic activities of leukocytes.

There are several kinds of in vitro assays for chemotactic activities. One of the most popular methods is the filter assay, using Boyden's chambers or transwells (14,15). Although it still remains as one of the standard methods for cell migration assay, it provides only limited level of information as the data is derived only from the proportional distribution of cells across the filter at the end of the experiment. In addition, it is theoretically impossible to discriminate chemotaxis from chemokinesis, because a single filter cannot produce a stable concentration gradient of chemokines.

Another is the filming method that traces the motion of cells by video or cinematography. It was as early as from 1930's that real cellular motion has been traced by microscopic cinematography (16-18). Since then, instrumentation is improved so much as optic as well as electronic technologies are advanced (15,19,20). The information provided by the filming method is much more abundant than that by the filter assay, including the locomotive pattern, morphological change and moving speed of the cells. However, one of the critical limits is that a stable concentration gradient is not provided in most of the filming methods. In this situation, it is possible to compare the cell morphology, migration speed and locomotive patterns before and after chemotactic stimulation, but not possible to observe and analyze the true sense of chemotaxis, that is, the directional migration depending on the concentration gradient of the chemotactic agents (21). Other assays such as those using Zigmond's chambers provide temporal concentration gradient of chemotactic agents, but they are restricted to only adherent cells (22). Very recently, a novel optical assay became available, that provides microchannel in which stable concentration gradients of chemokines can be maintained for a long time (20, 23). In the present study, for the first time, we have observed and analyzed the locomotive patterns of HL-60 cells in a stable concentration gradient of chemokines by using this new optical assay.

Materials and Methods

Culture of HL-60 cells. HL-60 cells (CCL240, American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI1640 supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 50 μg/ml streptomycin sulfate (Sigma, St. Louis, MO, USA), 50 units/ml penicillin G (Sigma), and 2 mM L-glutamine (Gibco, Gaithersburg, MD, USA). HL-60 cells (5.0×10^5 cells/ml) were stimulated by adding 0.8% DMF (Wako, Osaka, Japan) or 50 ng/ml PMA (Sigma). After 4 days, the cells were harvested for the analysis of morphology, flow cytometry and chemotactic responsiveness. When necessary, dead cells were removed before use by centrifugation on Ficoll-Hypaque (d=1.077, Pharmacia Biotech, Uppsala, Sweden) at $400 \times g$ for 30 minutes.

Purification of human peripheral blood granulocytes. Granulocyte fraction was purified for comparison from adult human peripheral bloods (PBs). Mononuclear cells (MNCs) were removed by density centrifugation on Ficoll-Hypaque, and the pellet was washed in phosphate-buffered saline (PBS, pH 7.4) and was resuspended in PBS. Six percent dextran T-500 (Pharmacia Biotech) in PBS was added to make the final concentration of 1%, and the cell suspension was allowed to stand at room temperature to let RBCs to settle down. After 1 hr, the supernatant was harvested and

Table I. Fluorescent antibodies used in flow cytometric analysis

Antibodies	Isotype	Clone	Fluorochrome	Manufacturer
Anti-hCD11c	Mouse IgG1/k	B-ly6	PE	BD Pharmingen
Anti-hCD18	Mouse IgG2a	1B4	FITC/PE	BD Pharmingen
Anti-hCD14	Mouse IgG2a/k	M5E2	FITC	BD Pharmingen
Anti-hCCR3	Rat IgG2a/k	61828	FITC/PE	R & D
Anti-hCCR5	Mouse IgG1	CTC5	PE	R & D
Anti-hCCR9	Mouse IgG2a	112509	FITC/Cy	R & D
Anti-h128B	Mouse IgG1/k	6C6	FITC	BD Pharmingen
Anti-hCXCR4	Rat IgG2a/k	1D9	PE	BD Pharmingen
Isotype-control	Mouse IgG1	MOPC-21	PE	BD Pharmingen
Isotype-control	Rat IgG2a/k	R35-95	PE	BD Pharmingen

was washed by centrifugation at 600×g for 10 minutes. The remained red cells were lysed by incubation in 0.17 M Tris-ammonium chloride (0.83%) solution for 5 min at 37°C. After being washed again, the cells were resuspended in chemotaxis medium.

Morphological analysis. For morphological analysis, the cells were spun at 50×g for 5 min onto glass slides (Cytospin 3, Shandon, Osaka, Japan). The slides were air-dried and stained with May-Grunwald-Giemsa solution. They were observed under a light microscope.

Flow cytometry. For flow cytometric analysis, the cells were blocked by incubation with 10% human AB^+ sera for 10 minutes, and were stained for 30 minutes with combination of the fluorescent antibodies (Table I). Annexin V conjugated with fluorescein isothiocyanate (FITC, Pharmingen, San Diego, CA) was also used for measurement of apoptosis. Cells were stained with 1 mM annexin V in 10 mM HEPES buffer (pH 7.4) containing 140 mM NaCl and 2.5 mM $CaCl_2$. Samples were analyzed on a FACSCalibur flow cytometer (BD, Mountain View, CA, USA). The data was analyzed by using CellQuest (BD) software.

Chemotaxis assay using transwell. HL-60 cells, unstimulated and stimulated with 0.8% DMF or 50 ng/ml PMA for 4 days, were washed and resuspended in RPMI 1640 containing 0.25% low endotoxin BSA (Sigma) (chemotaxis medium) in a cell density of 10^6 cells/ml. One hundred μl of the prepared cells were placed into the upper chambers of transwells (6.5 mm diameter, polycarbonate membranes with pore size of 5 μm and 8 μm for DMF-stimulated cells and unstimulated/PMA-stimulated cells, respectively, Corning Inc., Acton, MA, USA). Six hundred μl of chemotaxis medium with or without CXCL12/stromal derived factor-1 α (SDF-1 α), CXCL8/interleukin-8 (IL-8) and/or CCL3/macrophage inflammatory protein-1 α (MIP-1 α), were placed in the lower chambers. All the chemokines were purchased from R&D (Minneapolis, MN, USA) and were used at a final concentration of 20 ng/ml. After 4 hours at 37°C, migrating (lower chamber) cells were counted with the use of FACScalibur.

Chemotaxis assay using a time-lapse videomicroscopy. In vitro chemotactic activities of the cells were also analyzed by a time-lapse videomicroscopic technique using a special equipment (EZ-TAXIScan; Hirata Co., Tokyo, Japan)(20,23). The cells were applied into one compartment of a small visually accessible chemotactic chamber, consisting of a cover slip precoated with fibronectin (10 μg/ml, Invitrogen Co., Carlsbad, CA, USA) and an etched silicon chip, in which two com-

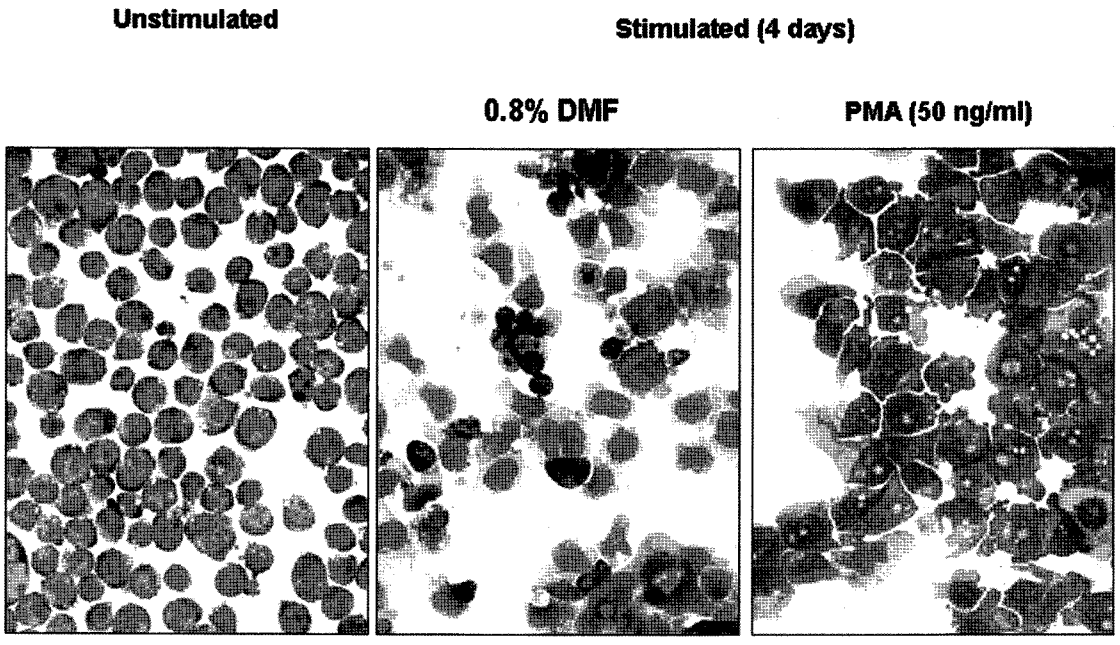

Figure 1. Stimulation with DMF or PMA induced morphological changes in HL-60 cells. Light microscopic observation of the May-Grunwald-Giemsa-stained preparations of HL-60 cells before and after stimulation with 0.8% dimethylformamide (DMF) or 50 ng/ml phorbol myristate acetate (PMA) for 4 days (×400). Unstimulated HL-60 cells show high N/C ratio, cytoplsmic granules, and 2 to 3 prominent nucleoli. In the DMF-stimulated HL-60 cell fraction, about 40~50% cells show granulocytic differentiation. Fully maturated neutrophils with segmented nuclei and azurophilic granules are observed. Small numbers of metamyelocyte-/myelocyte-like cells with cytoplasmic granules are also observed. In the PMA-stimulated cell fraction, almost all cells are large and show convoluted nuclei with relatively fine chromatins and abundant ground glassy cytoplasm with fine cytoplasmic granules.

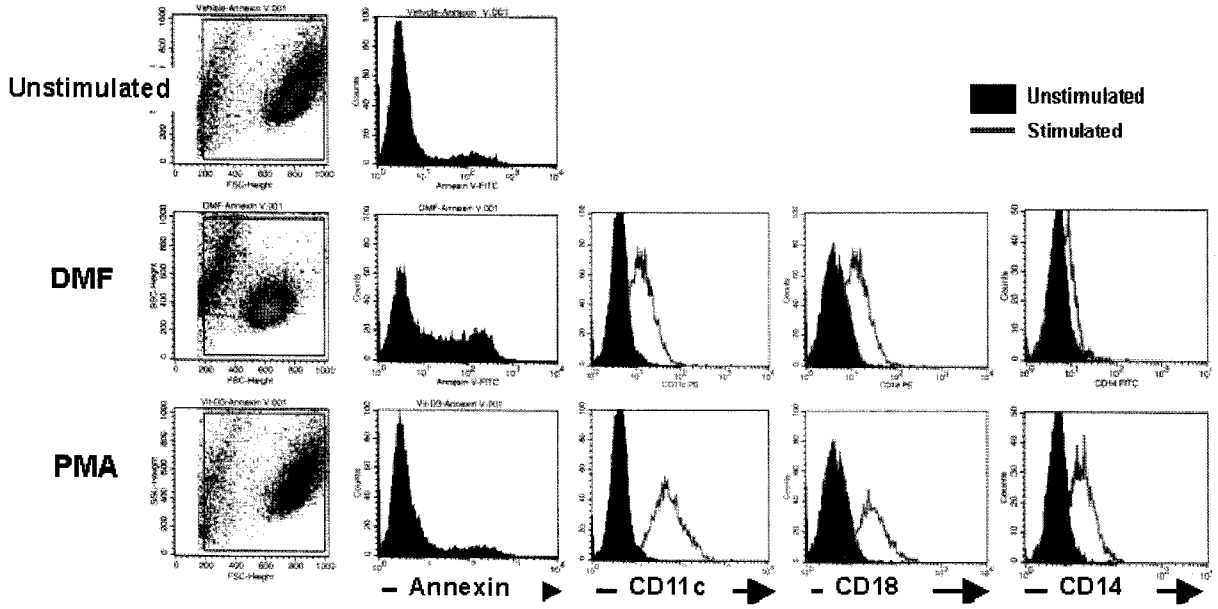

Figure 2. Stimulation with DMF or PMA induced phenotypic changes in HL-60 cells. Representative density plots and histograms for flow cytometric analysis of apoptosis and surface molecules on HL-60 cells before and after stimulation with 0.8% dimethylformamide (DMF) or 50 ng/ml phorbol myristate acetate (PMA). These plots are representatives of more than three separate experiments showing similar results.

partments are connected by a microchannel. Sometimes, before application, the cells were pretreated with pertussis toxin (100 ng/ml in 10 mM sodium phosphate buffer (pH 7.0) containing 50 mM NaCl; Calbiochem, San Diego, CA, USA) for 1 hr at 37°C. After addition of chemokines in the opposite compartment, cellular movements were recorded by taking serial photos at an appropriate interval (30～90 sec) for a sufficient time (60～360 min). After assay, the photo images were transformed into a movie file using an animation program (Ulead Photo Explorer version 7.0, Ulead Systems Inc., Tokyo, Japan). The locomotive pattern and moving speed were analyzed by drawing the trajectory of each cell using a digitizer. In addition, the number of cells that migrating into the assay field (toward the chemokine) was enumerated by using a special program, TAXIScan analyzer, provided by the manufacturer.

Statistical analysis. Data were represented as mean±SE of more than three separate experiments. Statistical significance was determined by using the Student t test. All comparisons were 2-tailed, and a P value less than 0.05 was considered significant.

Results

Stimulation with DMF or PMA induced morphological differentiation in HL-60 cells. Unstimulated and vehicle-stimulated HL-60 cells showed high N/C ratio, cytoplsmic granules, and 2 to 3 prominent nucleoli. These findings are consistent with neoplastic promyelocytes (Fig. 1). After stimulation with DMF for 4 days, about 40～50% cells showed granulocytic differentiation. Fully maturated neutrophils with segmented nuclei and azurophilic granules were observed. Small numbers of metamyelocyte-/myelocyte-like cells with cytoplasmic granules were also observed. In the PMA-stimulated cell fraction, almost all cells were large in size and showed convoluted nuclei with relatively fine chromatins and abundant ground glassy cytoplasm with fine cytoplasmic granules.

Stimulation with DMF or PMA induced phenotypic changes in HL-60 cells. Annexin-V staining showed that spontaneous apoptosis occurred in about 5～10% of unstimulated HL-60 cells (Fig. 2). Stimulation with PMA increased apoptosis just minutely but that with DMF significantly increased apoptosis in HL-60 cells. Stimulation with either DMF or PMA for 4 days increased the surface expression of CD11c and CD18 in HL-60 cells. However, the expression of CD14 was significantly increased only by stimulation with PMA.

Change in the expression of chemokine receptors in HL-60 cells before and after stimulation with DMF or PMA. Stimulation with PMA increased the surface expression of CCR3, CXCR2 and CXCR4 in HL-60 cells, whereas that with DMF did not except for CXCR2 (Fig. 3). Even in the cases of CCR5 and CXCR4, the expression levels seemed to be decreased by stimulation with DMF. However, in comparison with the isotype controls, CXCR4 was still highly, while CCR5 was

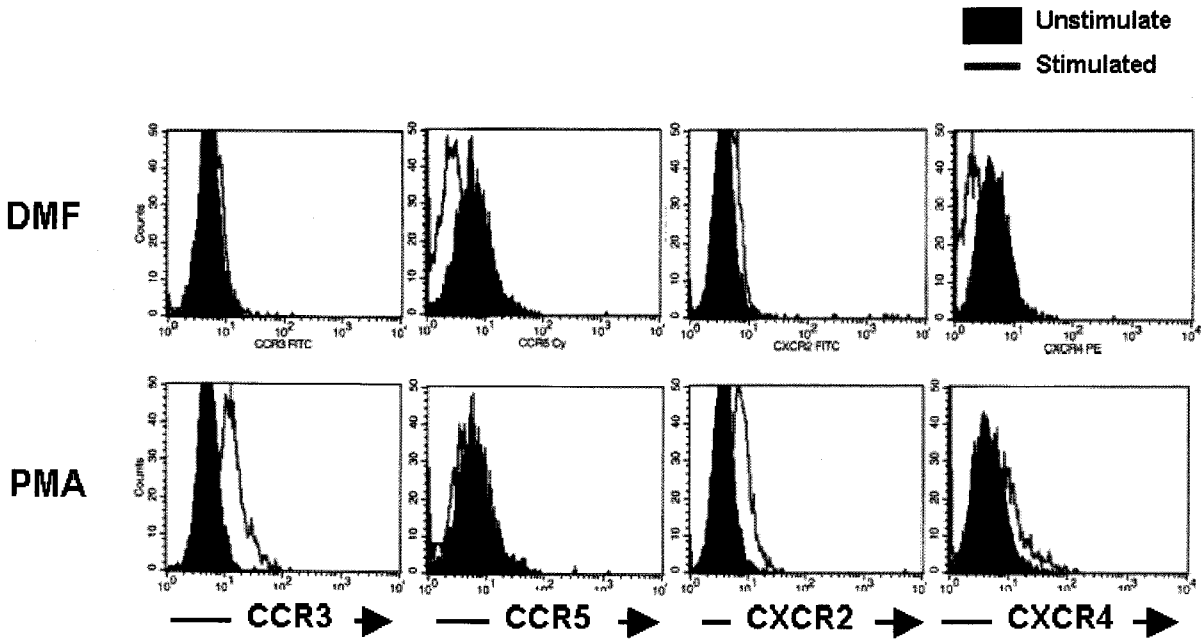

Figure 3. Stimulation with PMA increased the surface expression of CCR3, CXCR2 and CXCR4 in HL-60 cells, whereas that with DMF did not except for CXCR2. Representative histograms for flow cytometric analysis of surface expression of chemokine receptors on HL-60 cells before and after stimulation with dimethylformamide (DMF) or phorbol myristate acetate (PMA). These histograms are representatives of more than three separate experiments showing similar results.

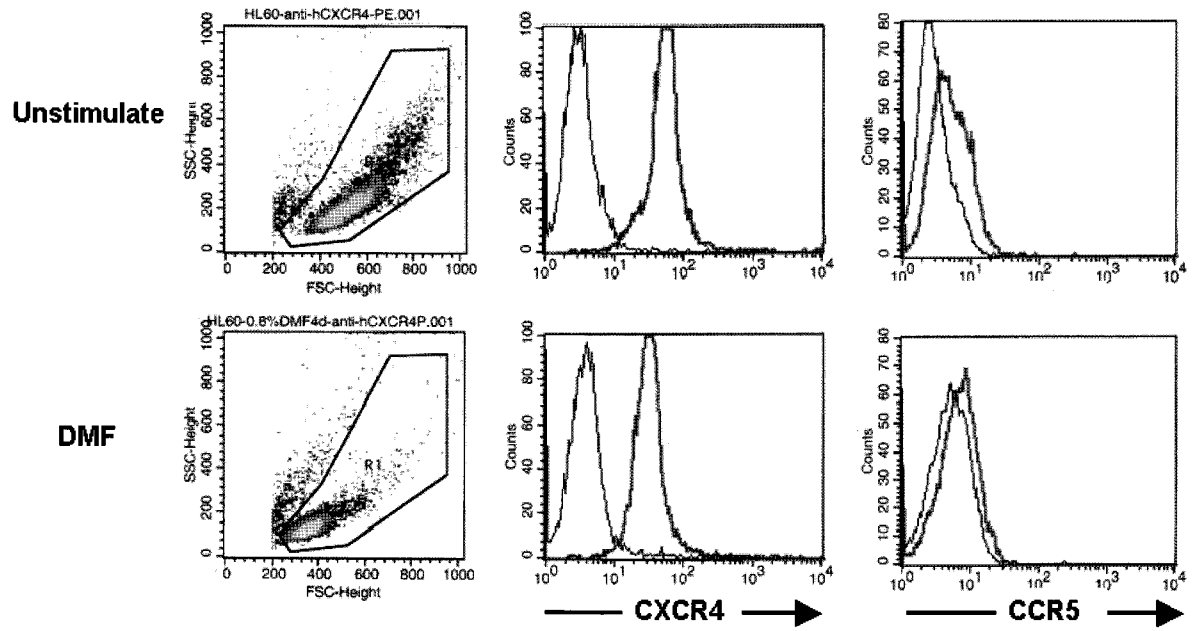

Figure 4. Although decreased, CXCR4 is still highly, while CCR3 is lowly but significantly, expressed on HL-60 cells stimulated with DMF. Representative histograms for flow cytometric analysis of surface expression of CXCR4 and CCR5 on HL-60 cells before and after stimulation with dimethylformamide (DMF). Thin lines represent isotype controls while the thick lines represent the specific antibodies. These histograms are representatives of more than three separate experiments showing similar results.

lowly but significantly, expressed on HL-60 cells stimulated with DMF (Fig. 4).

Transwell assay showed that chemotactic activities are induced in HL-60 cells after stimulation with DMF or PMA. Unstimulated HL-60 cells showed little (0.81 ± 0.05%) spontaneous migration in transwell assay (Fig. 5).

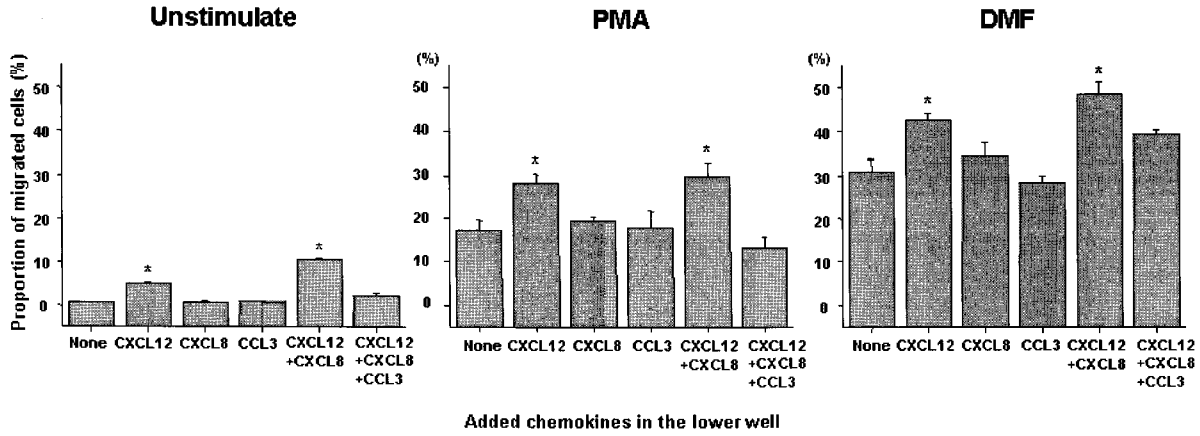

Figure 5. Transwell assay showed that chemotactic activities were induced in HL-60 cells after stimulation with dimethylformamide (DMF) or phorbol myristate acetate (PMA). HL-60 cells, unstimulated and stimulated with 0.8% DMF or 50 ng/ml PMA for 4 days, were placed into the upper chambers of transwells (6.5 mm diameter, polycarbonate membranes with pore size of 5 μm and 8 μm for DMF-stimulated cells and unstimulated/PMA-stimulated cells, respectively). The chemokines were added into the lower chambers. After 4 hours at 37°C, the proportions of migrating (lower chamber) cells were counted with the use of FACScalibur. *$p < 0.05$, compared with none.

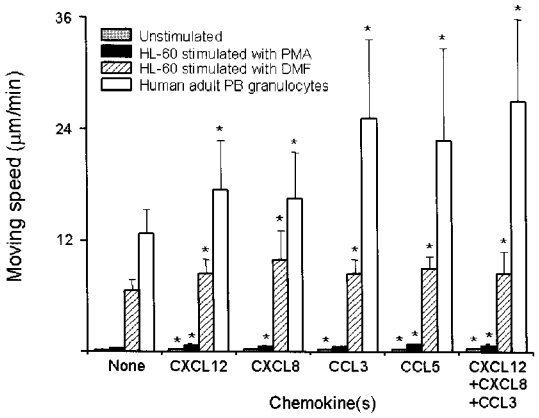

Figure 6. Moving speed of HL-60 cells and human peripheral blood (PB) granulocytes measured by a time-lapse videomicroscopy. The speed was calculated by dividing the distance of displacement estimated by tracing the trajectory of each cell with the time that elapsed. *$p < 0.05$, compared with none.

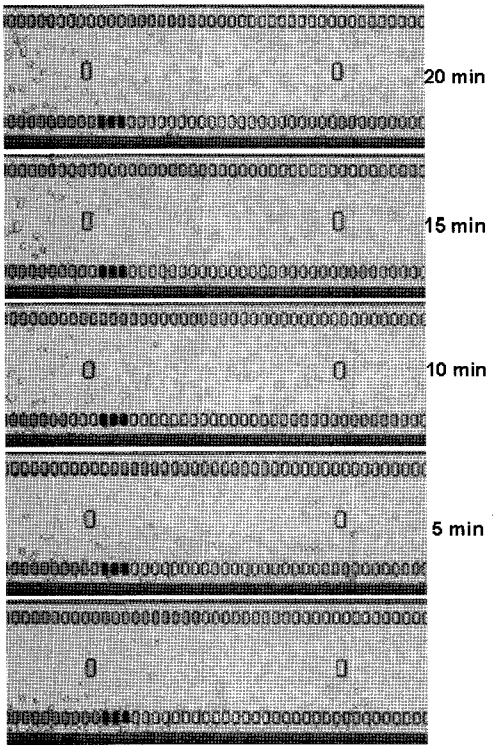

Figure 7. Directional migration of DMF-stimulated HL-60 cells in a microchannel in which a concentration gradient of CXCL12/SDF-1 α was created from the above to the bottom. The cells were applied into the lower and the chemokine into the upper compartment, respectively.

CXCL12 increased the migrated proportion of unstimulated HL-60 cells slightly but significantly (4.70±0.28%). On the other hand, in PMA- and DMF-stimulated HL-60 cells, substantial proportions of cells migrated spontaneously without any chemokine stimulation (18.4±2.18% and 31.0±2.76%, respectively). CXCL12 alone or in combination with CXCL8 also significantly increased the migrated proportions in PMA- and DMF-stimulated HL-60 cells. However, CXCL8 alone or in combination with CCL3 did not significantly increase the migrated proportions.

Locomotive patterns and chemotactic activities of HL-60 cells observed by the time-lapse videomicroscopic assay. Visual observation of the real motion revealed that unstimulated HL-60 cells showed no directional movements but just tiny oscillations even in a chemokine milieu

(Fig. 6). Although the extent of oscillation increased significantly by several kinds of chemokines, true sense of space displacement was not observed in unstimulated HL-60 cells. By contrast, DMF-stimulated cells showed vigorous random migration even in a milieu deficient of any chemokines (Fig. 6, 8). When they are moving, they become rapidly and continuously polarized protruding clearly distinguishable fronts and tails. The speed of movement was calculated by dividing the distance of displacement estimated by tracing the trajectory of each cell with the time that elapsed. The spontaneously moving speed of the DMF-stimulated HL-60 cells in a milieu deficient of chemokines is $6.99 \pm 1.24\,\mu m/min$ (n=100). When they are placed in a milieu with concentration gradient of chemokines, the moving speed was significantly increased (Fig. 6) and they moved directionally toward the chemokine while still wandering very much (Fig. 7, 8). The directional movements of the DMF-stimulated HL-60 cells were almost completely abolished by pretreatment of the cells with pertussis toxin, demonstrating the $G\alpha_i$-mediated processes (Fig. 9). To summarize, DMF-stimulated HL-60 cells showed vigorous spontaneous movement as well chemotactic responses but their moving speeds were about half the level of human PB granulocytes (Fig. 6). On the other hand, PMA-stimulated HL-60 cells did not show any true sense of space displacement. Their locomotive patterns were very similar to, although their motions were a little larger than, those of unstimulated HL-60 cells (Fig. 6).

Discussion

Observation of the real cellular motions in a stable concentration gradient of chemokines revealed that HL-60 cells develop chemotactic activities as they are differentiated into granulocytes by stimulation with DMF. Undifferentiated HL-60 cells did not show true sense of chemotaxis as they did not induce any space displacement or morphological polarization even in a chemokine milieu. However, by the conventional method of transwell assay, undifferentiated HL-60 cells seemed to show a little but significant chemotactic response to CXCL12 alone or, in particular, in combination with CXCL8 (Fig. 5). The discrepancy between the videomicroscopy and transwell assay in the interpretation of chemotactic activities of undifferentiated HL-60 cells may originate from the mechanism of the assays themselves. As one of the prototype of filter assay, transwell assay counts the number of cells that migrate across the single membrane filter. Although undifferentiated HL-60 cells showed just tiny oscillation without true space displacement, the minute increase in the extent of oscillation indu-

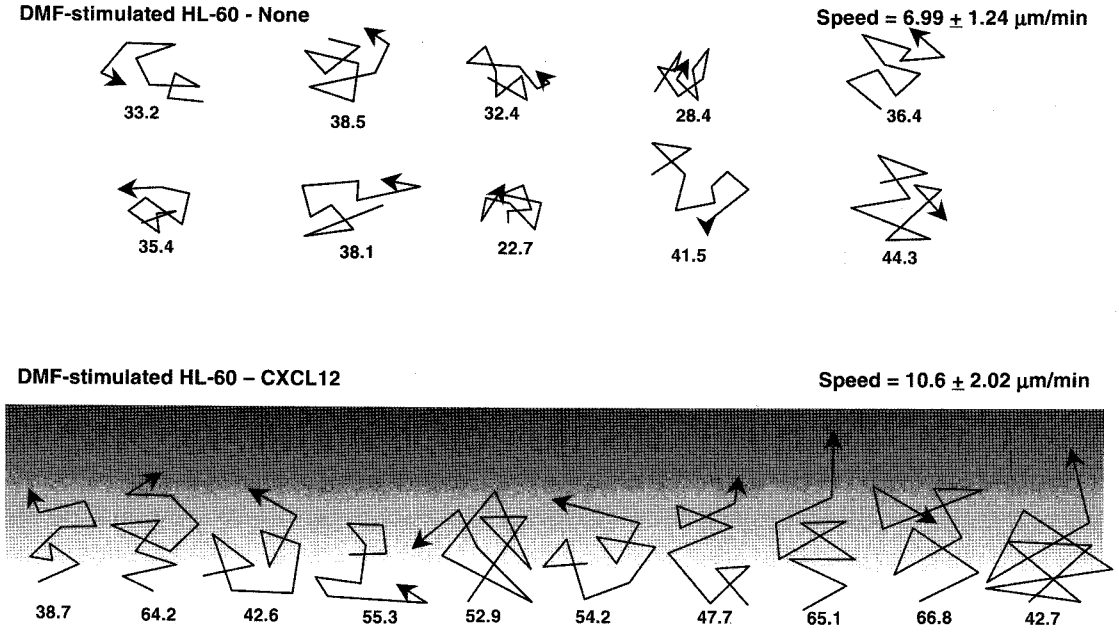

Figure 8. Locomotive patterns of DMF-stimulated HL-60 cells in a milieu without (upper) or with a concentration gradient of chemokine (CXCL12) (lower). In a milieu deficient of any chemokine, DMF-stimulated HL-60 cells spontaneously moved vigorously but randomly (upper). In a milieu where a concentration gradient of CXCL12 is created from the above to the bottom, the locomotive patterns of DMF-stimulated HL-60 cells changed so that they began to move directionally toward CXCL12 although they still wandered very much and the moving speed was significantly increased (lower). Representative trajectories of randomly selected 10 cells traced by using a digitizer during 10 frames (300 sec) of the optical assay. Numbers represent the distance of cell movement in μm during the 300 sec.

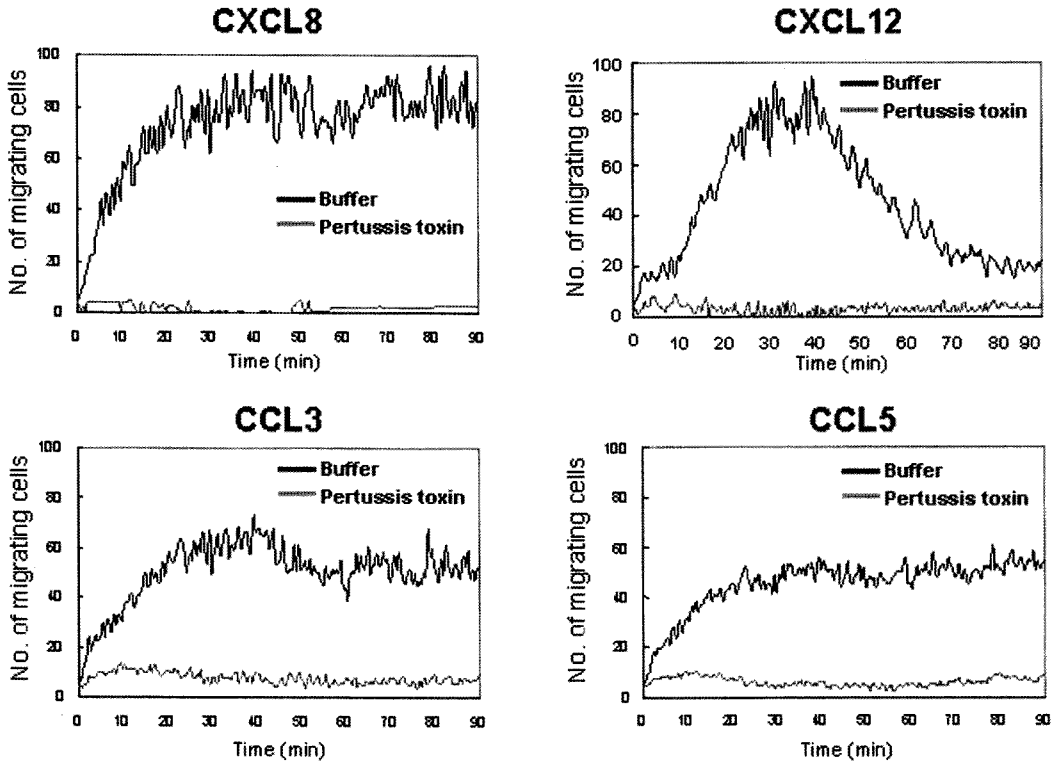

Figure 9. Directional migration of DMF-stimulated HL-60 cells in response to the chemokines, CXCL8, CXCL12, CCL3, and CCL5, were abolished by pretreatment with pertussis toxin, demonstrating that they are G α i-mediated chemotactic responses. DMF-stimulated HL-60 cells, pretreated with pertussis toxin (100 ng/ml) or buffer, were placed in a microchannel where a concentration gradient of the indicated chemokine was created, and the number of migrating cells toward the chemokine was automatically counted using the software, TAXIScan analyzer.

ced by stimulation with the chemokines may be reflected as the significant increase in migrating proportions in the transwell assay.

On the contrary, DMF-stimulated HL-60 cells seemed not to show chemotactic response to CXCL8 or CCL3 by transwell assay, but they did by videomicroscopic assay. This discrepancy may be due to the intrinsic motility of the DMF-stimulated HL-60 cells. As they spontaneously move vigorously (Fig. 6, 8), the basal level of spontaneously migrating proportion is already very high and the chemotactic indices induced by chemotactic stimulation cannot be high enough by transwell assay (Fig. 5). In this case, the pore size of the transwell membrane may be a critical factor (24), and transwells with the pore size of 5 μm were used in the present study for the sake of fair comparison because the same size of microchannel (height, 5 μm) was used in the videomicroscopic assay. For reference, transwells with a larger (8 μm) pore size were used for unstimulated as well as for PMA-stimulated HL-60 cells.

On the other hand, any kinds of stimuli that can increase the cellular motility irrespective of the directionality of the movement can be interpreted as inducing chemotactic response in transwell assay. In these cases, visual observation of the real cellular motions in a chemokine milieu is very helpful to appropriately decide the chemotactic activities of the cells. Meanwhile, PMA-stimulated HL-60 cells did not show any directional migration in response to chemotactic stimulation by the videomicroscopic observation (Fig. 6), but they did show chemotactic response to CXCL12 alone as well as in combination with CXCL8 by transwell assay (Fig. 5). Compared with the undifferentiated HL-60 cells, not only the spontaneously migrating proportions but also the chemotactic indices were remarkably increased in the PMA-stimulated HL-60 cells. Nevertheless, any directional movements were not observed by videomicroscopic assay. In this case, it seems reasonable to suspect the possibility that true chemotactic response may not be observed by the videomicroscopy. Videomicroscopy itself provides the means of observing the real cellular motions and has no reason to interfere with the cellular movements, but the microenvironment for the cellular movements employed in the videomicroscopic assay may be critical. In fact, the core technology of the videomicroscopic assay

employed in the present study is the small visually accessible chemotactic chamber, KK microchamber, consisting of a cover slip and an etched silicon chip, that had been proved to provide a stable concentration gradient of chemokines for a long time (23). The microenvironment in the microchamber for the chemotactic assay can be created conveniently by precoating the cover slips with the extracellular matrices (ECMs) and/or any other bound molecules that had been chosen according to the purpose of experiment. In the present study, we have coated the cover slips with fibronectin, which is a multifunctional glycoprotein found both in plasma and ECMs, mediating cell adhesion to ECMs (25). Fibronectin is involved in various biological events including cell proliferation, embryonic development, wound healing, and disease pathogenesis (26). On the other hand, fibronectin molecule is also known to be highly susceptible to proteolytic degradation by inflammatory proteinases such as elastase, cathepsin, and matrix metalloproteinases (27). Although known as one of the best ECMs for cell migration assay supporting many kinds of cells to migrate (28), fibronectin alone may not be sufficient to support the migration of PMA-stimulated HL-60 cells, as they do migrate on FBS-coated cover slips (data not shown). However, FBS cannot be a defined microenvironment as it contains so many kinds of ECM proteins and, in addition, various kinds of chemotactic agents. Accordingly, it still remains to be investigated to search for the optimum microenvironment that can support the migration of PMA-stimulated HL-60 cells.

Visual observation of the real cellular motions revealed the locomotive patterns of the granulocytic cells in vitro. DMF-stimulated HL-60 cells showed vigorous spontaneous migration just like PB granulocytes even in the absence of chemokines, implying that granulocytic cells are intrinsically motile. The granulocytic cells showed directional migration in a stable concentration gradient, but they still wandered very much. This kind of locomotive pattern was not different, although the moving speed was somewhat different, according to the type of chemokine. Therefore, wandering seems to be a characteristic locomotive pattern of granulocytic cells.

Neutrophils are short-lived, terminally differentiated cells and cannot be genetically manipulated. In order to study neutrophils in vitro, blood should be drawn every time. It would be very convenient if cell line models are available that can simulate neutrophil functions, and differentiated HL-60 is one of the most commonly used ones (7). Concerning the chemotactic activities, the locomotive pattern of DMF-stimulated HL-60 cells is almost the same as that of neutrophils. Although the moving speeds of DMF-stimulated HL-60 cells are about half of those of neutrophils (Fig. 6), they seem to be enough for most kinds of quantitative assays. Consequently, DMF-stimulated HL-60 cells may be a convenient model for quantitative chemotactic assays, in particular, in combination with the time-lapse videomicroscopy.

In the present study, stimulation with PMA increased the surface expression of CCR3, CXCR2 and CXCR4 in HL-60 cells, whereas that with DMF did not increase, instead decreased the expression of CCR5 and CXCR4 (Fig. 3). However, DMF-stimulated HL-60 cells showed vigorous directional migration in response to the CCR5- and CXCR4- ligands, CCL3 or CCL5 and CXCL12, respectively. Sometimes, the expression of chemokine receptor and functional chemotactic responsiveness may be discrepant (29), in particular, in the cases of redundant ligand-receptor combination (30). One of the examples is the combination of CCR5 with CCL3 or CCL5, as CCR5 can bind many other ligands including CCL4, CCL8 and CCL16. However, only one ligand, CXCL12, has been discovered for CXCR4 so far (31). One possibility to explain such discrepancies is that responsiveness to a chemokine such as CXCL12 is determined by a factor(s) other than the receptor expression level, as has been suggested previously (32).

In summary, the time-lapse videomicroscopic assay using the KK microchamber demonstrated that HL-60 cells developed chemotactic activities to inflammatory chemokines (CCL3, CCL5 and CXCL8) as well as a homeostatic chemokine (CXCL12) during differentiation into granulocytes by stimulation with DMF. DMF-stimulated HL-60 cells may be a convenient model for chemotactic assays, in particular, in combination with the time-lapse videomicroscopy, providing a valuable tool for the studies on neutrophil chemotaxis.

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