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Activated adherent large granular lymphocytes/natural killer (LGL/NK) cells change their migratory behaviour

A. PIRELLI, P. ALLAVENA & A. MANTOVANI *Laboratory of Human Immunology, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy*

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SUMMARY

Unstimulated large granular lymphocytes/natural killer (LGL/NK) cells, unlike small T lymphocytes, exhibit prompt locomotion into nitrocellulose filters in response to chemo-attactrants, but, unlike monocytes, are unable to migrate as adherent cells across polycarbonate filters. Upon activation with 4-B-phorbol 12,13 dibutyrate (PDBU), LGL/NK cells become adherent and change their migratory behaviour, having the ability to migrate as adherent cells across polycarbonate filters. PDBU-treated high-density T lymphocytes did not show, under the same conditions, locomotory activity. The change in migratory behaviour following activation may represent an important determinant of the ability of activated LGL/NK cells to adhere to vascular linings and localize in tissues.

Cells with the morphology of large granular lymphocytes (LGL) and with natural killer activity (NK) constitute a minor, but appreciable, proportion of circulating mononuclear cells (Herberman & Ortaldo, 1981; Trinchieri & Perussia, 1984). LGL/NK cells have typical morphology and cytochemistry, express a specific set of monoclonal antibody-defined membrane structures and, in addition to NK activity, mediate antibody-dependent cellular cytotoxicity (ADCC).

LGL/NK cells are likely to play an important role as a first mechanism of resistance against foreign cells, microbes and possibly tumours (Herberman & Ortaldo, 1981; Trinchieri & Perussia, 1984). The regulation of the recruitment of LGL/NK cells from the blood compartment is probably an important factor determining the expression of NK function in normal and diseased tissues.

In an effort to investigate LGL/NK cells recruitment, we have developed an *in vitro* assay for the migration of these cells based on the use of nitrocellulose filters. We and others found that LGL/NK cells exhibit prompt *in vitro* migration in response to chemo-attractants (Bottazzi *et al.*, 1985; Polentarutti *et al.*, 1986; Pohajdak *et al.*, 1986; Natuk & Welsh, 1987), an observation consistent with the view that these cells represent a first, easily mobilizable, line of resistance.

Activated NK cells have long been known to exhibit increased adherence (Tai & Warner, 1980), and phorbol esters, potent activators of the cytotoxic function of NK cells, have been reported to induce selective adherence of LGL/NK cells (Argov *et al.*, 1985). We therefore examined whether PDBU altered the migratory properties of LGL/NK cells.

Correspondence: Dr P. Allavena, Laboratory of Human Immunology, Istituto di Recherche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milan, Italy.

Table 1 shows a representative experiment, demonstrating the migratory behaviour of LGL using polycarbonate and nitrocellulose filters. While monocytes migrated across polycarbonate and nitrocellulose filters equally well, only the latter were found suitable for resting LGL/NK cells to migrate in reponse to chemo-attractants. The failure of resting LGL to migrate across polycarbonate filters is presumably related to the fact that in the absence of activation they do not have adherent properties: locomotion through nitrocellulose filters does not involve adhesion to a substratum, whereas migration across polycarbonate filters requires adhesion (Wilkinson, Haston & Shields, 1982).

When LGL/NK cells were treated with 100 nM PDBU, they acquired the ability to migrate as adherent cells across polycarbonate filter, even in the absence of chemo-attractants. Eight experiments have been performed with similar results.

When activated serum (a source of C5a) was seeded in the lower compartment of chemotaxis chambers as chemo-attractant, a modest augmentation of PDBU-treated LGL migration occurred.

Small lymphocytes (predominantly T cells) isolated from high-density Percoll fraction did not have appreciable locomotory activity under these conditions, with only occasional cells being counted in polycarbonate filter after PDBU treatment (Table 1).

As shown in Fig. 1, optimal experimental conditions for LGL/NK cells activation were observed with 100 nM of PDBU. Small T lymphocytes were not stimulated by PDBU treatment at any concentration level. As already found for nitrocellulose filters, LGL/NK cell migration was better observed after 2 hr, as after 1 hr only few cells had migrated.

The results presented here demonstrate that PDBU-activated LGL/NK cells change their migratory behaviour and

Table 1. Migration of phorbol ester-treated adherent LGL/NK cells through different filters

Cells treated with	Cells	No. of migrated cells (polycarbonate filters)		Migrated distance (nitrocellulose filters)		MI
		Medium	Activated serum	Medium	Activated serum	
—	High-density T lymphocytes	Undetectable	Undetectable	45·1 ± 4	52 ± 3·1	7
	Large granular lymphocytes	Undetectable	Undetectable	63·1 ± 3·2	83·2 ± 3·9*	20
	Monocytes	43 ± 2	330 ± 3*	46·2 ± 1·2	75 ± 24*	29
PDBU 100 n*M*	High-density T lymphocytes	Undetectable	Undetectable	Undetectable	Undetectable	—
	Large granular lymphocytes	59 ± 5	78 ± 1*	67·9 ± 0·4	80·2 ± 0·9*	12

Purified preparations of human peripheral blood LGL/NK cells were obtained by density Percoll gradient (Pharmacia Uppsala, Sweden) as described by Timonen & Saksela (1980), with minor modifications (Bottazzi *et al.*, 1985). Cell preparations usually contained 70–90% LGL, as morphologically identified in Giemsa-stained cytopreps, and less than 2% monocytes. Locomotory activity of LGL/NK cells, high-density T lymphocytes (bottom of Percoll gradient) and Percoll-isolated monocytes (Colotta *et al.*, 1984) was evaluated in a chemotaxis assay using a microchamber technique and two different filters (Bottazzi *et al.*, 1985). With 5-μm pore-size polycarbonate filters (Nucleopore Corp. Pleasanton, CA), chemotaxis was evaluated as the number of cells migrated through the filters. Five oil immersion microscope fields were counted: results are expressed as mean of three replicates ± SD.

In other experiments 8 μm porous nitrocellulose filters were used (Sartorius GmbH, Göttingen, FRG). with these filters, chemotaxis was evaluated as the distance (in μm) migrated by the two leading cells or as a migration index, which represents the difference between migration with and without the stimulus (zymosan-activated serum, used at 5%).

LGL/NK cells and T lymphocytes were resuspended in the upper part of the chemotaxis chamber at 2×10^6/ml in growth medium (RPMI-1640 + 10% bovine serum; Gibco, Grand Island, NY) or in medium with 100 nM 4-B-phorbol 12,13 dibutyrate (PDBU; Sigma, St Louis, MO). Incubation time of locomotion assay was 2 hr.

* $P < 0.05$ versus medium.

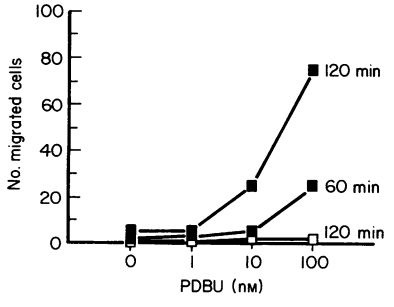

Figure 1. Locomotory activity of activated LGL/NK cells: dose–response curve with PDBU and timing of chemotaxis assay with policarbonate filters. LGL/NK cells (■) and small T lymphocytes (□) were seeded in the upper compartment of a chemotaxis microchamber with different concentrations of PDBU. Results are presented as number of cells migrated through the policarbonate filter after 60 or 120 min.

acquire the ability to migrate as adherent cells through polycarbonate filters. That activated LGL/NK cells have an increased propensity to adhere has been shown repeatedly. In particular PDBU, an activator of cytotoxic function (Ramos, Masucci & Klein, 1985; Trinchieri *et al.*, 1984), was shown to induce preferential adherence of LGL/NK cells (Argov *et al.*, 1985), and was therefore selected for the present study. While this report was being prepared, Vujanovic *et al.* (1988) reported that IL-2-activated NK cells rapidly became adherent to plastic. In preliminary experiments, (not shown) we found migration as adherent cells in polycarbonate filters of IL-2-activated LGL, although the proportion of IL-2-activated cells adhering to filters and migrating was variable from one experiment to the other. These results indicate that the alteration of migratory behaviour may represent an important change in the properties of NK cells following activation by physiological or pharmacological stimuli.

We have reported previously, using the nitrocellulose filter system, that various NK cells stimulants augment the penetration of LGL/NK cells into filters but that stimulated LGL/NK cells are relatively unresponsive to a gradient of chemotactic agents (Polentarutti *et al.*, 1986). This observation was confirmed here studying the migration of adherent LGL/NK cells in polycarbonate filters. The passage of PDBU-treated (2 hr) cells across polycarbonate pores was only modestly augmented by seeding a chemo-attactant in the lower compartment of the chamber. Since exposure to phorbol ester results in phosphokinase C depletion, the unresponsiveness to chemo-attractants of activated NK cells may reflect exhaustion of this signal transduction mechanism.

The alteration of migratory potential detected by this *in vitro* model system in activated NK cells may have *in vivo* relevance. The acquisition by LGL of the capacity to adhere and migrate as adherent cells may be important for adhesion to endothelial linings and extravasation in tissues.

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