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Possible Chemotaxis of Human Monocytes by *N*-Acetylmuramyl-L-Alanyl-D-Isoglutamine

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N-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP) induced the chemotaxis of human blood monocytes without mediation of serum complement. Specificity (dependency on chemical structure) of the chemotactic activity of MDP was evidenced by a negligible activity of analogs lacking most of the immunomodulating activities of MDP.

Recently we reported that bacterial cell walls, peptidoglycans, and fragments of peptidoglycan, enzymatically obtained or synthesized, stimulated the migration of human monocytes (6a).

In the present study, the checkerboard analysis described by Zigmond and Hirsch (10) was made to see whether the stimulation of monocyte migration by *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is caused by chemotaxis, in which the increased migration is directed by the concentration gradient of the molecule, or by chemokinesis, in which random migration of cells is simply enhanced. MDP and its adjuvant-inactive analogs were synthesized as described previously (5). *N*-formylmethionyl-leucyl-phenylalanine (FMLP) (Sigma Chemical Co., St. Louis, Mo.) was dissolved in *N*,*N*-dimethylformamide (Wako Pure Chemical Industries, Osaka, Japan) at a concentration 10^{-2} M and was stored at −20°C until use as a reference chemotactic agent. MDP, its analogs, and FMLP was dissolved and diluted with the medium previously described (Gey BSA-GVB^{2+}, reference 6a) to appropriate concentrations (3, 7) and used for chemotaxis assay.

Heparinized venous blood drawn from healthy donors was fractionated by a modification of the Ficoll-Hypaque method (2) to obtain a cell fraction which, on the average, consisted of 20% monocytes and 80% lymphocytes. Cells were washed with phosphate-buffered saline (PBS, pH 7.0) and suspended at a density of 5×10^6 cells/ml of Gey BSA (Gey balanced salt solution, 2% bovine serum albumin, 20 mM HEPES). Assays were performed as described before (6a), using a multiwell chemotaxis assembly (1, 3, 4, 7). Briefly, each well on a bottom plate was filled with 25 μl of a test or a reference solution, a polycarbonate filter sheet of 5-μm pore size was placed on the bottom plate, and a gasket and a top plate were fixed in place. Fifty microliters of the cell suspension was added to each well on the top plate. The whole assembly was incubated at 37°C for 90 min in the humidified air containing 5% CO_2.

After the incubation, the filter was removed, fixed, and stained with Diff-Quick (International Reagents Co., Kobe, Japan), which enabled easy distinction between monocytes and lymphocytes on the basis of cell morphology. Microscopic counts were made of the number of monocytes which had completely passed through the filter. Data were expressed as the mean ± standard error of the number of monocytes per oil immersion field.

Figure 1 shows that MDP significantly enhanced monocyte migration at concentrations ranging from 0.001 to 1 μg/ml, although the extent of the increase of migration was less than that caused by FMLP. In contrast, MDP analogs, whose D-isoglutamine residue was replaced by L-isoglutamine, D-glutamine, D-glutamic acid, L-glutamic acid, or D-isoasparagine, were completely inactive. This finding indicates that the monocyte migration-enhancing activity of MDP is highly dependent upon the chemical structure of the molecule.

To determine whether the observed increase of monocyte migration caused by MDP is merely because of increased chemokinesis or directed migration toward a positive stimulus (chemotaxis), various concentrations of MDP were added to either the upper well, the lower well, or both wells in a checkerboard pattern (Table 1), and the monocyte migrations in these wells were compared. The assay result strongly suggests that the increased monocyte migration caused by MDP is directed by the concentration gradient of MDP, i.e., mainly due to chemotaxis. Strictly speaking, the chemotactic assembly used here, in which the filter sheet is only 10 μm thick, makes it impossible to estimate the distance of monocyte migration in the filter, so that evaluation of the enhancement of migration due

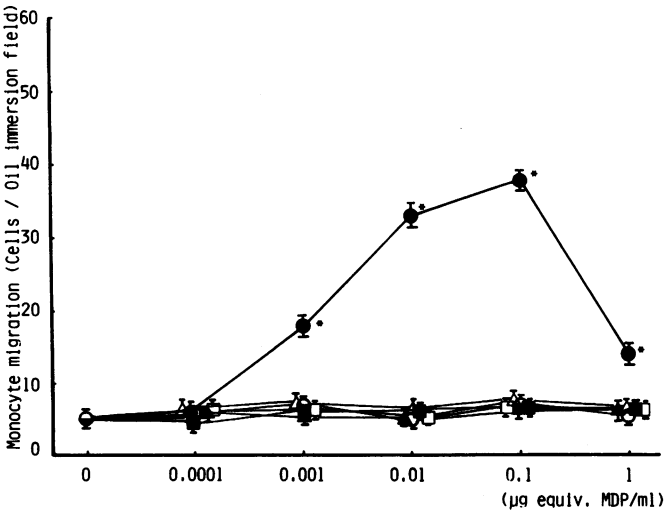

FIG. 1. Monocyte migration induced by MDP (●) and its adjuvant-inactive analogs: MurNAc-L-Ala-L-isoGln (○); MurNAc-L-Ala-D-Gln (□); MurNAc-L-Ala-D-Glu (▲); MurNAc-L-Ala-L-Glu (■); MurNAc-L-Ala-D-isoAsn (△). Data represent the mean ± standard error of the number of monocytes per oil immersion field in an experiment performed at the same time. (Triplicate filters were submitted to assay of each dose of each specimen.) The value of a positive control (FMLP, 10^{-8} M) was 52 ± 3, and that of negative control (⊖) was 5 ± 1. *, Significantly different from a negative control with neither test nor reference specimen ($P < 0.01$).

to chemotaxis was by calculation according to the formula of Zigmond and Hirsch (10). Therefore, confirmation by direct microscopic visualization of the orientation behavior of the monocytes treated with MDP is needed to reach the final conclusion.

It should be emphasized that the stimulation of chemotaxis by MDP was almost certainly due to the direct action of the molecule, but not to chemotactic mediators produced by activation of complement system, since the assay system used in this study was not supplemented with extraneous serum complement. The possibility of the involvement of cell-derived chemotactic factors in the observed increase of monocyte migration may be excluded by the fact that even if cell-derived chemotactic factors were produced by MDP which diffused into the upper well holding monocytes and lymphocytes, the concentration gradient of these factors between the upper and lower wells could not explain the increased migration of monocytes through the filter toward the lower well.

TABLE 1. Dependence of monocyte migration on MDP concentration gradient[a]

Concn (μg/ml) of MDP in lower well	Cells/O.I.F. ± SE[b] at concn (μg/ml) of MDP in upper well:			
	0	0.001	0.01	0.1
0	**4 ± 1**[c]	6 ± 1	4 ± 1	4 ± 1
0.001	5 ± 1	**2 ± 1**	3 ± 1	5 ± 0
0.01	24 ± 1	13 ± 1	**5 ± 1**	2 ± 1
0.1	33 ± 1	27 ± 3	5 ± 1	**7 ± 1**

[a] MDP was added to both wells or either of the lower and upper wells of the multiwell chemotaxis chamber at indicated concentrations. Monocytes were added to the upper well.

[b] The extent of monocyte migration was determined as described in the text. The number of monocytes per oil immersion field (O.I.F.) on the lower surface of a polycarbonate filter was triplicately counted on 20 microscopic fields to obtain the mean ± standard error.

[c] Boldface numbers indicate those values obtained when the concentrations of chemoattractant on both sides of the filter were identical.

MDP, the minimum effective structure responsible for the majority of multifold immunomodulating activities of bacterial cell walls, has been shown to stimulate macrophages and monocytes, the cells which play the very important roles in natural and acquired host defense mechanisms, in various ways (8). The present study has added a new item to a list of macrophage and monocyte-stimulating activities of MDP. The MDP induction of monocyte chemotaxis may seemingly be in conflict with MDP inhibition of macrophage migration in a capillary tube assay (6, 9). However, this seeming contradiction may not be a real one, but may be due to the different assay conditions. It seems that the enhanced chemotaxis and the inhibited migration are different expressions of stimulated macrophage functions.

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