Changes in Phenotype of Monocyte-Like THP-1 Cells Associated with Transendothelial Migration

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The level of expression of CD11b and HLA-DR surface molecules on monocyte-like THP-1 cells increased significantly as a result of transmigration of these cells through a monolayer of endothelial cells. The expression of all studied markers (CD11b, HLA-DR, and CD-14) increased significantly after transendothelial migration in the presence of TNF-α and IFN-γ. The changes in surface phenotype of THP-1 cells after transendothelial migration in the presence of TNF-α were more pronounced than in the presence of IFN-γ. Transendothelial migration of THP-1 cells in the presence of IL-4 caused less pronounced changes in the surface phenotype, which did not differ from changes in transmigration without cytokines.

Key Words: *mononuclear phagocytes; transendothelial migration; cytokines*

Leukocytes incessantly migrate from the blood into tissues in order to realize their functions [2]. The properties of leukocytes are modified during migration into the subendothelial space. For example, the expression of activation markers, costimulatory and adhesion molecules, proliferative and cytotoxic activities, and viability of migrating cells increase [5]. Monocytes after migration into tissues differentiate into macrophages, myeloid dendritic cells, osteoclasts, microglia cells, and perform numerous functions aimed at homeostasis maintenance [6].

We studied the effect of transendothelial migration on the surface phenotype of mononuclear phagocytes. The process of transendothelial migration of monocytes is regulated by many endogenous and exogenous factors, by cytokines, among other things [3,4]. We studied the phenotypical changes in monocyte-like THP-1 cells after transendothelial migration through a monolayer of endothelial cells (EA.hy 926 human continuous cells) in the presence of TNF-α, IFN-γ, and IL-4 cytokines released by the immune system cells at different stages of inflammation and immune response.

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MATERIALS AND METHODS

EA.hy 926 human cells were a kind gift from Dr. Cora-Jean C. Edgell (University of North California). The cells were cultured in DMEM/F12 (Biolot) with appropriate additives at 37°C in a humid atmosphere with 5% CO_2. For monolayer disintegration, the cells were incubated in Versene (Biolot) for 5-10 min. Human monocyte-like THP-1 cells were cultured in RPMI-1640 (Biolot) with additives in a humid atmosphere with 5% CO_2 at 37°C. The cells were reinoculated every 3-4 days. The cells of all strains used in the study were certified. Cell viability in all experiments was at least 98%.

The following recombinant preparations of human cytokines were used: TNF-α, refnolin (specific activity 1 U 0.06 ng) in a concentration of 50 U/ml; IFN-γ, gammaferone (Ferment Company, Sanitas) in a concentration of 500 U/ml; IL-4 (Becton Dickinson; specific activity 1 U 0.2 ng) in a concentration of 50 U/ml. The cytokines in these concentrations exhibited no cytotoxic effects towards cells used in the study.

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Endothelial EA.hy 926 cells were put into transwells (Becton Dickinson) with 8-μ pores (Fig. 1) in a concentration of 50,000 cells in 150 μl complete DMEM/F12 (Sigma) and incubated at 37°C in a humid atmosphere with 5% CO_2 until the formation of a confluent monolayer, which was evaluated under a microscope. THP-1 cells (30,000 in 150 μl DMEM/F12 with 1.5% FCS, ICN) were added into the upper transwell compartments (above the endothelial cell monolayer). The medium (350 μl of the same DMEM/F12; Sigma) with or without cytokines was put into the lower compartments. In parallel experiments, THP-1 cells were put into the upper compartments of the transwells containing no endothelial cells, with or without cytokines in the lower compartment.

After 72-h incubation at 37°C in a humid atmosphere with 5% CO_2, the entire contents of the lower compartment was collected and the expression of surface molecules was evaluated. Anti-HLA-DR, anti-CD11b labeled with PE, FITC-labeled polyclonal antibodies to mouse immunoglobulins (Med-BioSpektr Company), and anti-CD14 labeled with PerCP (Becton Dickinson) were added to cells and incubated according to manufactirer's recommendations. Fluorescence intensity was evaluated on a FACSCalibur cytofluorometer (Becton Dickinson). The results were expressed as the mean fluorescence intensities (MFI) estimated by the results of at least 3 independent experiments. The means were compared using Student's *t* test.

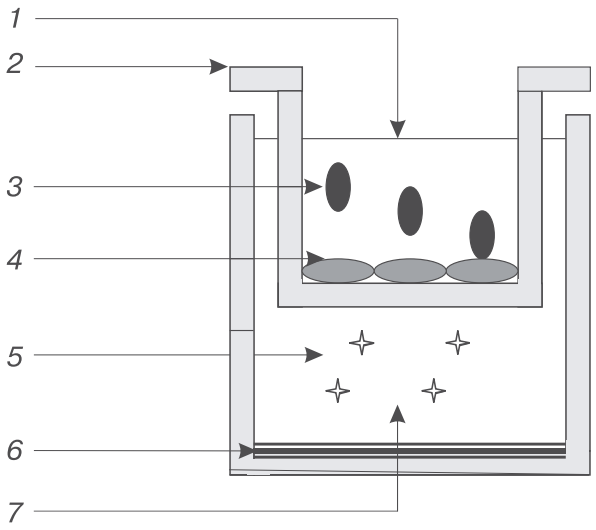

Fig. 1. Scheme of experiment. *1*) upper compartment; *2*) transwell; *3*) THP-1 cells; *4*) EA.hy 926 cells; *5*) cytokines; *6*) gelatin; *7*) lower compartment.

RESULTS

The effect of transendothelial migration on mononuclear phagocyte differentiation was evaluated by comparing the phenotype of THP-1 cells migrating from the upper compartment of the transwell into the lower one through a monolayer of endothelial cells with the phenotype of THP-1 cells migrating from the transwell upper compartment to the lower one without passing through endotheliocyte monolayer. The expression of CD11b and HLA-DR molecules on THP-1 cells increased significantly only after transendothelial migration, while CD14 expression remained unchanged (Table 1). In order to confirm that the observed phenotypical changes resulted from transendothelial migration, we additionally evaluated the phenotype of THP-1 cells remaining in the upper compartment of the transwell above the endotheliocyte monolayer (which were in contact with endothelial cells, but did not trans-

TABLE 1. Changes in Expression of Surface Molecules on THP-1 Cells after Transendothelial Migration (*M*±*m*)

Cell incubation in the lower compartment	CD11b		HLA-DR		CD14	
	I	II	I	II	I	II
With						
medium	14.90±2.45 (*n*=6)	24.30±8.71* (*n*=8)	50.40±5.17 (*n*=7)	68.40±19.27* (*n*=7)	8.20±2.56 (*n*=5)	12.90±5.87 (*n*=7)
TNF-α, 50 U/ml	12.00±3.44 (*n*=6)	62.3±24.2*** (*n*=8)	48.10±7.85 (*n*=5)	129.1±35.5*** (*n*=9)	10.30±4.61 (*n*=5)	102.3±5.8** (*n*=8)
IFN-γ, 500 U/ml	29.8±12.0 (*n*=4)	60.20±10.12** (*n*=4)	70.00±16.42 (*n*=6)	113.9±35.6* (*n*=6)	13.20±4.23 (*n*=4)	22.70±1.99* (*n*=4)
IL-4, 50 U/ml	13.30±3.34 (*n*=4)	31.30±8.35** (*n*=4)	44.67±3.66 (*n*=4)	85.17±18.30** (*n*=6)	12.20±0.76 (*n*=4)	16.30±4.65 (*n*=4)

Note. Here and in Table 2: results are expressed in fluorescence intensity units minus control for second antibodies or isotypical control. *I*: THP-1 cells not passing through endothelial cell monolayer; *II*: THP-1 cells passing through endothelial cell monolayer. ***$p<0.001$, **$p<0.01$, *$p<0.05$ compared to *I*.

TABLE 2. Expression of Surface Molecules on THP-1 Cells Migrating in the Presence of Cytokines and without Cytokines in the Lower Compartment (*M*±*m*)

Cell incubation in the lower compartment	CD11b	HLA-DR	CD14
With			
TNF-α, 500 U/ml	12.00±3.44 (*n*=6)	48.10±7.85 (*n*=5)	10.30±4.61 (*n*=5)
IFN-γ, 500 U/ml	29.8±12.0* (*n*=4)	70.00±16.42* (*n*=6)	13.20±4.23 (*n*=4)
IL-4, 50 U/ml	13.30±3.34 (*n*=4)	44.67±3.66 (*n*=4)	12.20±0.76 (*n*=4)
medium without cytokines	14.90±2.45 (*n*=6)	50.40±5.17 (*n*=7)	8.20±2.56 (*n*=5)

migrate). No appreciable differences in the expression of surface molecules on THP-1 cells of this fraction in comparison with that on intact THP-1 cells were detected.

Changes in the surface phenotype of THP-1 cells after transendothelial migration could result from contact and/or distant interactions with endothelial cells. Hence, we studied the relationship between phenotypical changes in transendothelial migration and the endotheliocyte secretory and adhesion characteristics. According to published data and our previous findings, TNF-α, IFN-γ, and IL-4 cytokines have different effects on secretory and adhesion characteristics of endotheliocytes [1, 3,4]. We compared the phenotype of THP-1 cells after their passage through endotheliocyte monolayer in the presence of TNF-α, IFN-γ, and IL-4 with the phenotype of THP-1 cells migrating into the lower compartment in the presence of the same cytokines without passing through endotheliocyte monolayer. A significant increase in the expression of all studied markers (CD11b, HLA-DR, and CD14) was observed only after transendothelial migration in the presence of TNF-α and IFN-γ (Table 1).

Changes in the transendothelial migration in the presence of TNF-α were more pronounced than those in the presence of IFN-γ. Transendothelial migration in the presence of IL-4 caused a significant increase in the expression of CD11b and HLA-DR, while the level of CD14 expression virtually did not differ from the expression of this molecule on THP-1 cells migrated without passing the endotheliocyte monolayer in the presence of IL-4 in the lower compartment.

The cytokines modified not only endothelial, but also THP-1 cells reacting with them. For this reason, we compared the phenotype of THP-1 cells, which migrated from the upper to the lower compartment in the presence of cytokines without passing the endotheliocyte monolayer, with the phenotype of THP-1 cells migrated without cytokines in the lower compartment. Changes in the phenotype were negligible and significant only in the presence of IFN-γ (Table 2).

Differentiation of mononuclear phagocytes is one of the key events in the development of inflammation and immune response. Our results indicate that the expression of surface markers associated with differentiation on monocyte-like THP-1 cells migrating into the subendothelial space increased after transendothelial migration. These changes were more pronounced in case of transendothelial migration in the presence of proinflammatory cytokines, which can be explained by higher sensitivity of cells migrating into the subendothelial space to cytokines. Our experiments confirmed the important contribution of endothelial cells to regulation of mononuclear phagocyte differentiation.

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