Induction of LFA-1-mediated homotypic adhesions in promonocytic U-937 cells occurs independently of cell differentiation

Carlos Cabañas 1, Pedro Lastres 1, Teresa Bellón 1, Patricio Aller 1, Carl G. Figdor 2, Angel Corbi 3 and Carmelo Bernabeu 1

1 Centro de Investigaciones Biológicas, C.S.I.C., Madrid (Spain), 2 The Netherlands Cancer Institute, Amsterdam (The Netherlands) and 3 Servicio de Inmunología, Hospital de La Princesa-UAM, Madrid (Spain)

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The differentiation of monocytes into macrophages occurs along with a marked increase in LFA-1-dependent intercellular adhesions. Similarly, the phorbol ester-induced differentiation of U-937 promonocytic cells into macrophage-like cells is morphologically characterized by an important increase in LFA-1/ICAM-1-dependent intercellular homotypic adhesions. Since an important functional role in activation of human T cells has been demonstrated for LFA-1-dependent adherence, we have analyzed whether the induction of LFA-1-dependent intercellular adhesion of human monocytic cells is necessarily accompanied by differentiation of these cells. We found that treatment of the promonocytic U-937 cells with the anti-LFA-1 mAb NKI-L16 induces formation of intercellular clusters, but does not induce cell differentiation as determined by several differentiation markers. These markers include the arrest of cell proliferation, production of reactive oxygen species, changes in the cell surface expression of differentiation-associated antigens such as the transferrin receptor, CD11b and CD11c and changes in the levels of several specific gene transcripts such as CD18 antigen, c-myc, ornithine decarboxylase and vimentin. These findings suggest that LFA-1-dependent adhesion and differentiation of monocytic cells are independent processes.

Introduction

In mononuclear phagocytes, the regulation of cell-cell adhesion is an important aspect of their function and development. Thus, monocytes initiate diapedesis by adherence to the endothelium, extravasate and mature into tissue macrophages. In addition, upon antigen challenge macrophages infiltrate into the site of inflammation and display homotypic (macrophage-macrophage) and heterotypic (macrophage-T cell) interactions. These cellular associations can lead to granuloma formation in certain diseases (lepromatous leprosy, tuberculosis, sarcoidosis, etc.) [1]. When monocytes are exposed in vitro to immunologic or inflammatory stimuli such as interferon gamma, LPS or TNF, an induction of homotypic adhesions occurs concomitantly with activation and differentiation of these cells [2–4]. Similarly, phorbol esters induce cellular differentiation as well as an increase in intercellular adhesions, as evidenced by cell cluster formation, in the human promonocytic U-937 cells and in the myelomonocytic cell line HL-60 [5–10]. This adhesion of monocytic cells is mediated by the LFA-1 and ICAM-1 molecules [10,11]. Since binding of ligand to LFA-1 is required for T cell function [12] and the existence of regulatory signals conveyed by LFA-1 has been suggested for T lymphocytes [13,14], we have analyzed in this report the possible occurrence of cell differentiation in the human promonocytic U-937 cells when these cells are induced to aggregate via LFA-1/ICAM-1 by a mAb specific for LFA-1.

Materials and Methods

Antibodies

The mAb NKI-L16 (anti-LFA-1 α or CD11a) induces LFA-1/ICAM-1 mediated homotypic interac-
tions [15]. The mAb FG1/6 (anti-transferrin receptor or CD71) [16], Bearl (anti-CD11b) [17] and HCl/1 (anti-CD11c) [18] were generated in our laboratories. Antibodies were of the IgGl (FG1/6, HCl/1 and Bearl) and IgG2a (NKI-L16) subclasses. Antibody NKI-L16 was added to cell cultures as 1:1000 dilution of ascites fluid.

**Cells**

The promonocytic U-937 and the myelomonocytic HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 ng/ml) (Flow Laboratories, Rockwell, MD) in a 5% CO₂ atmosphere at 37°C. When necessary, cells were incubated with either NKI-L16 mAb (1:1000 dilution of ascites fluid) or 10 ng/ml of PMA for 24-48 h to induce intercellular cluster formation or cell differentiation.

**Markers of monocytic differentiation**

Cellular proliferation was measured as previously described [19]. Flow cytometry analysis was performed with an EPICS-CS flow cytometer (Coulter Cientifica, Mostoles, Spain) using logarithmic amplifiers. For antigen expression analysis, cells were incubated with the corresponding mAb for 30 min at 4°C. After two washes with NaCl/Pi (140 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄ and 8 mM KH₂PO₄, pH 7.4), FITC-labeled rabbit anti-mouse IgGl (Nordic Immunology, Tilburg, Denmark) were added and incubation followed by an additional period of 30 min at 4°C. Finally, cells were washed twice with NaCl/Pi and their fluorescence estimated.

Determination of generation of hydrogen peroxide was carried out as reported by Bass et al. [20]. Briefly, U-937 cells were loaded with the nonfluorescent compound 2',7'-dichlorofluorescein diacetate (DCFH-DA), which is deacetylated by cellular esterases into DCFH. Then, the oxidation of DCFH by the stimulus-induced generation of hydrogen peroxide results in the fluorescent compound DCF which is detected by flow cytometry.

RNA blot analyses were performed basically as described [19]. The probes used were: the 1.5 kb ClaI-EcoRI fragment of pMC413rC plasmid [21], which contains the 3rd exon of human c-myc; the 1.1 kb human vimentin-specific XhoI fragment of LzA₁A plasmid [22]; the 1.5 kb OrnDC-specific XhoI fragment of OD821 plasmid (a generous gift from Dr. L. Kaczmareck, Varsow, Poland); and the 1 kb EcoRI fragment from the cDNA of the human CD18 antigen [23].

**Results**

The addition of the anti-LFA-1 mAb NKI-L16 to U-937 cultures results in an important induction of intercellular homotypic adhesions evidenced by formation of large cell clusters which remain in suspension in the culture medium (Fig. 1). These aggregates are induced as soon as 2 h after the addition of the mAb and the cells remain clustered for up to 3 days. A similar effect on cluster formation is induced by treatment of U-937 cells with the phorbol ester PMA. However, this PMA-induced aggregation of U-937 cells is somehow different from that induced by NKI-L16, since it is not observable before 15-20 h of treatment with PMA and is accompanied by an important increase in cellular adhesiveness to the plastic substrate. Both, NKI-L16 and PMA induced cell aggregates are LFA-1/ICAM-1-mediated, since mAb to either of these ligands are able to inhibit cluster formation.

To address the question of whether this LFA-1-mediated aggregation of U-937 cells induced by the mAb NKI-L16 is also accompanied by induction of cell differentiation, as is the case for PMA, we have analyzed the changes in several myeloid differentiation markers. Proliferation of U-937 cells completely ceases upon induction of differentiation into macrophage-like cells with PMA (Table I). On the contrary, the addition of the mAb NKI-L16 had no effect on U-937 proliferation.

![Fig. 1](image-url)
TABLE I

Effect of homotypic adhesions induced by the NKI-L16 mAb on the proliferation of U937 cells

U937 cells were cultured in the absence or in the presence of either PMA or NKI-L16 mAb. The [³H]-thymidine uptake was measured as described in Materials and Methods. The mAb 8F3 (anti-CD13) was included as a control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[³H]Thymidine uptake (cpm)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>72682</td>
</tr>
<tr>
<td>PMA</td>
<td>1102</td>
</tr>
<tr>
<td>NKI-L16</td>
<td>64453</td>
</tr>
<tr>
<td>8F3</td>
<td>65571</td>
</tr>
</tbody>
</table>

Fig. 2 shows the changes in the cell surface expression of CD11b, CD11c and CD71 antigens during the PMA-induced differentiation of U-937 cells. An important increase in CD11b and CD11c expression and the loss of CD71 characterize the differentiation of U-937 into macrophage-like cells [18]. However, the induction of intercellular homotypic adhesion induced by NKI-L16 is not accompanied by any of the aforementioned antigenic changes.

Also, the differentiation of U-937 cells induced with PMA is characterized by an important decrease in the levels of c-myc and OrnDC as well as the marked increase of the levels of CD18 and vimentin transcripts [19,24]. In contrast, the treatment of U-937 cells with NKI-L16 has no effect on the levels of any of these gene transcripts (Fig. 3).

Finally, the differentiation of U-937 into macrophage-like cells is functionally characterized by the acquisition of capacity to generate reactive oxygen derivatives. Fig. 4 shows that PMA-differentiated U-937 cells but not NKI-L16-treated cells are able to generate hydrogen peroxide upon stimulation with high doses of PMA.

Taken together, these results suggest that LFA-1-dependent homotypic adhesions are not sufficient to induce monocyctic differentiation of U937 cells.

Discussion

Interactions of monocytes with endothelial cells and with a variety of cell types and matrices occur concomitantly with their differentiation into tissue macrophages. The importance in this process of the CD11/CD18-dependent interactions is evidenced in CD18 immunodeficient patients by the paucity of phagocytes at the site of inflammation [25]. Using the human promonocytic cell line U937 as an experimental model of differentiation, we show that extensive LFA-1-ICAM-1-dependent interactions can occur in the absence of monocytic differentiation. This conclusion is supported by the findings that (a) PMA is able to differentiate U937 cells into macrophage-like cells in the absence of LFA-1/ICAM-1-dependent cellular interactions [19]; and (b) in CD18 deficient patients, LFA-1 adhesion independent functions of phagocytes are all normal, including shape changes, binding of chemotactic compounds (FMLP) and oxygen radical generation to soluble stimuli [26].

Our results using monocyctic cells do not support the existence of LFA-1-mediated transductive signals as
Fig. 4. Effect of NKI-L16 induced homotypic adhesions on the generation of reactive oxygen species. U-937 cells were incubated in the absence or in the presence of either PMA or the mAb NKI-L16 for 48 h. Then, cells were loaded with DCFH-DA followed by stimulation with PMA and the fluorescence of the DCF generated was estimated by flow cytometry using logarithmic amplifiers. Cells treated with hydrogen peroxide (500 μM) were included as a positive control. Numbers at the upper right corner indicate the mean fluorescence intensity.

reported for T cells [13,14]. On the contrary, we think that the NKI-L16 mAb affects the conformation of LFA-1, thereby increasing the affinity of ICAM-1 without the need for signal transduction [27]. On the other hand, we can not exclude the requirement for other types of cell–cell and cell–matrix interactions during the monocytic differentiation process. In this sense, the monocytic antigen VLA-4 is able to interact with the endothelial molecule VCAM-1 [28]. Furthermore, the endothelial antigens CD62 and ELAM-1 and the homing receptor MEL-14 present in monocytes, are able to interact with the corresponding counterstructures (so far unknown) to allow monocyte-endothelial cell adhesions [29]. The possible implication of these molecules in the differentiation process needs to be elucidated.

Acknowledgements

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