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Differentiation of a Human Myeloid Cell Line (HL-60) Toward Granulocyte- and Macrophage-like Cells: Comparison of Cell Surface Antigen Expression

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A. Introduction

Human hematopoietic pathways have been defined mainly by the analysis of cellular morphological changes because of the paucity of other meaningful markers. Monocolonal antibodies against human cell surface antigens offer the possibility of following changes in the expression of these molecules, in particular when combined with the use of cell lines able to differentiate in vitro if provided with an appropriate stimulus. The aim of this work was to correlate antigenic changes on the surface of differentiating myelomonocytoid cells with the disappearance or appearance of morphologically distinct cell types during hematopoietic differentiation. The promyelocytic leukemia-derived cell line HL-60 was employed as an effective model system. This cell line [4] can be induced with retinoic acid to differentiate toward granulocytes [3], and after the addition of the phorbol ester TPA to HL-60 cells, they become macrophage-like [8]. The expression of surface antigens on these cells following the different types of induction treatment was analyzed with a panel of over 70 monoclonal antibodies [12], using indirect immunofluorescence and bacterial binding assays.

B. Materials and Methods

HL-60 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, antibiotics, and glutamine. They were induced with 1 μM retinoic acid, and the antigenic changes accompanying differentiation toward mature granulocyte-like cells were analyzed for 6 days by indirect immunofluorescence techniques [11] employing a panel of monoclonal antibodies, of which representative examples are show in Table 1. Simultaneously the morphology of cells was determined with cytocentrifuge preparations.

Induction of HL-60 cells toward macrophage-like cells was performed with 160 nM TPA, and the differentiating cells were characterized for 3–4 days by indirect bacterial binding assays [12].

C. Results and Discussion

The approach outlined here has made it possible to identify a number of antigenic determinants which are expressed to different degrees on the cell surface of myeloid and monocytoid cells, depending on the state of cellular differentiation. The results from the different types of induction experiments show that surface antigens on differentiating HL-60 cells can be assigned to at least seven categories (Table 2). (1) Antigens are not detectable on any of these cells (e.g., certain B-cell antigens like TÜ1). (2) Antigens are present on all cells irrespective of differentiation pathway and extent of differentiation (e.g., tissue common antigens and HLA-A, C heavy chains). (3) Both types of induction lead to the loss of antigens from the cell surface (e.g., the TÜ12 and TÜ15 antigens). (4) Only certain cell types appearing after inductions bear the antigenic determinants (e.g., M1/70.HL antigen). (5) Induction toward granulocytes leads to a higher percentage of antigen-

Table 1. Monoclonal antibodies and their specificity

Monoclonal antibody	Antigen or cell type detected	References
W6/32.HL	HLA-A, B, C, heavy chains	[2]
W6/32.HK	Inactive variant	[13]
TÜ48	HLA-Aw23, -Aw24, -Aw32, -Bw4	[5]
TÜ1	B-cell subpopulation, dendritic reticulum cells	[14]
TÜ3	Myeloid cells } different antigens	[10]
TÜ6, TÜ9	Myeloid cells } different antigens	[9]
OKT4	T-helper/inducer cells	[7]
TÜ12	T-cell subset, immature myeloid cells	[10]
TÜ15	Macrophages, immature myeloid, some T, B cells	[11]
M1/70.HL	Mac-1	[1]
TÜ28	Leukocyte subset	[10]
TÜ42	Leukocyte subset, different from TÜ28	[10]

Table 2. Expression of cell surface antigens on differentiating HL-60 cells

Monoclonal antibody or antigen detected	Induction treatment	
	RA[a]	TPA[b]
W6/32.HL	→[c]	→
TÜ48	→	↓
TÜ6, TÜ9, and other myeloid antigens	→	↓
OKT4	→	↓
TÜ12, TÜ15	↓	↓
M1/70.HL	↑	↑
TÜ3	↑	↓
TÜ28	⌒	→
TÜ42	↓ later ↑	↓

[a] RA, retinoic acid; expression determined by indirect immunofluorescence test

[b] TPA, 12-0-tetradecanoyl-phorbol-13-acetate; expression determined by bacterial binding assay

[c] "→" antigenic determinant expressed on an approximately equal percentage of cells during differentiation; "↓" antigenic determinant lost during differentiation; "↑" antigenic determinant appears on a larger fraction of cells during differentiation; "⌒" antigenic determinant expressed during differentiation only on certain cell types; further differentiation causes the loss of this determinant

bearing cells while differentiation towards macrophages causes the loss of the molecule from the cells (TÜ3) antigen). (6) Antigens present on certain cell types of uninduced and retinoic acid induced HL-60 cultures disappear from the cell surface after exposure to TPA (e.g., the myeloid TÜ6 or TÜ9 antigens, HLA-B molecules as detected by TÜ48, as well as the the antigen recognized by OKT4). (7) Antigens show an expression different from those described above (e.g., the TÜ28 or TÜ42 antigens).

It was a surprise to find that HL-60 cells reacted with OKT4, which has been described to be specific for T-helper cells [7], but several other antigens predominantly directed against T cells, like TÜ12 for example, also exhibit activity toward HL-60 cells. The loss of reactivity of TPA-induced cells with TÜ48 simultaneous with retention of HLA heavy chains as detected by W6/32.HL suggests that HLA-B antigens are under separate genetic control. This interesting phenomenon deserves further analysis (see also Ziegler et al., this volume). Table 2 shows that both differentiation pathways lead to the expression of a defined, easily distinguishable set of antigenic determinants, as defined by the panel of monoclonal antibodies employed here. A correlation of antigenic changes with morphologically distinct cell types reveals,

after both types of induction, that antigenic determinants which are lost after induction appear to be absent from more mature cell types while the reverse seems to be true for those antigens which are not expressed on uninduced HL-60 cells. Furthermore, most alterations in the expression of membrane antigens precede morphological maturation. Preliminary experiments with human bone marrow cells indicate that normal cell types exhibit changes in surface antigen phenotype and morphology comparable to those described here for an in vitro model system. Space does not allow a comparison of the results presented here with those of other investigators, but, e.g., Perussia et al. [6] have also used a panel of antibodies for similar purposes.

Further studies with HL-60 cells should be designed to answer questions regarding the molecular mechanisms involved in the regulation of differentiation-related gene expression.

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