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Hybrids Between Human Cell Lines Belonging to Different Hematopoietic Pathways: Analysis of HLA and Myeloid Surface Antigens

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A. Introduction

The control of gene regulation can be examined by somatic cell hybridization. Hybrids between human cell lines belonging to different hematopoietic lineages provide model systems for the analysis of the mechanisms governing the expression of cell surface antigens specific for a particular differentiation pathway [3, 4, 12]. In this study, the expression of antigens encoded by the human major histocompatibility (HLA) complex and of molecules present on myeloid cell types were analyzed with monoclonal antibodies on two somatic cell hybrids, HP-1 and PUTKO1.

B. Materials and Methods

HP-1 [4] was produced by fusing the Burkitt's lymphoma-derived B-cell line P_3HR-1 [3] and HL-60 [2], which is a promyelocytic leukemia-derived cell line. PUTKO was a somatic hybrid obtained by fusing P_3HR-1 and K562 [5], a fetal erythroid cell line [8]. All five cell lines were grown in tissue culture medium containing 10% fetal calf serum and antibiotics. Most of the monoclonal antibodies employed in this work have been described before (see Table 1). The expression of cell surface antigens recognized by monoclonal antibodies was determined using bacterial binding assays [11, 15].

Table 1. Monoclonal antibodies and their specificities

Monoclonal antibodies	Cell type or antigen detected	References
W6/32.HL	HLA-A, B, C heavy chains	[1]
W6/32.HK	Inactive variant	[13]
TÜ48	HLA-Aw23, -Aw24, -Aw32, -Bw4	[6]
2BC4	HLA-Bw6	Westphal, unpublished
TÜ22, TÜ34, TÜ35, TÜ36, TÜ37, TÜ39, TÜ43, TÜ58,	Ia-like antigens	[15]
YD1/63. HLK		[7]
TÜ3, TÜ50, TÜ51	Myeloid cells	[11]
TÜ5, TÜ6, TÜ9	Myeloid cells	[9]
TÜ8	Myeloid, some monocytoid and certain T and B cells	[11]
TÜ12	T-cell subset, immature myeloid cells	[11]

C. Results and Discussion

I. Antigens Encoded by the HLA Complex

All cell lines examined here expressed HLA heavy chains as detected by W6/32.HL (Table 2), a finding in line with previous results [3, 4, 12, 14]. The supertypic antigenic determinant HLA-Bw4, defined by TÜ48, was present on HL-60, P_3HR-1, and their hybrid HP-1 but lacking from K562 and the K562 × P_3HR-1 hydrid PUTKO1. An analysis of HLA antigen expression on DUTKO1, another K562 × B cell hybrid [12, 15] also indicated that K562 and hybrids derived from it have a deficiency in the expression of HLA-B antigens. These results make it likely that HLA-A,C, and HLA-B molecules are under separate genetic control. This situation seems to apply also to thymic cells, since HLA-B molecules are not detectable on cortical thymocytes, although these can be shown to express to other type(s) of HLA heavy chains (Müller et al., unpublished).

Table 2. Expression of major histocompatibility complex-controlled antigens by the hybrids and their parental cells

Antigen detected by	Cell line				
	HL-60	HP-1	P_3HR-1	PUTKO1	K562
W6/32.HL	100%[a], ~80[b]	100%, ~55	100%, ~60	80%, ~20	95%, ~15
W6/32.HK	–	–	–	–	–
TÜ48	100%, ~80	100%, ~30	100%, ~50	–	–
2BC4	NT[c]	NT	NT	–	–
TÜ22	2%, ~5	65%, ~20	78%, ~20	–	–
TÜ34	–	100%, ~40	98%, ~35	–	–
TÜ35	6%, ~5	94%, ~25	91%, ~35	≪1%, ~20	–
TÜ36	–	98%, ~40	94%, ~35	1%, ~50	–
TÜ37	–	99%, ~40	85%, ~25	<1%, ~30	–
TÜ39	7%, ~5	95%, ~25	92%, ~35	1%, ~30	–
TÜ43	–	100%, ~40	91%, ~35	<1%, ~20	–
TÜ58	2%, ~5	100%, ~40	86%, ~25	<1%, ~25	–
YD1/63.HLK	–	100%, ~30	73%, ~40	NT	–

[a] Percentage of cells with three or more bacteria bound
[b] Average number of bacteria bound per cell
[c] NT not tested

Table 3. Expression of "myeloid" antigens by the hybrids and their parental cells

Antigen detected by	Cell line				
	HL-60	HP-1	PUT	PUTK01	K562
TÜ3	35%[a],~ 10[b]	–	–	–	–
TÜ5	90%, ~ 60	–	–	–	–
TÜ6	90%, ~ 70	–	–	–	–
TÜ8	98%, ~ 90	21%, ~30	–	–	8%, ~ 9
TÜ9	98%, ~100	26%, ~30	–	–	19%, ~35
TÜ12	68%, ~ 15	40%, ~10	–	–	–
TÜ50	92%, ~ 70	9%, ~10	–	–	–
TÜ51	92%, ~ 90	–	–	–	–

[a] Percentage of cells with three or more bacteria bound
[b] Average number of bacteria bound per cell

In P_3HR-1 hybrids, whether the cells express Ia-like antigens seems to depend on the fusion partner. These molecules were present on virtually all HP-1 cells (with the exception of TÜ22 molecules), and could be detected even on a very small subpopulation of PUTKO1 cells. The postulated "dominance" of the K562 genome in a K562×B cell hybrid [3] is thus not complete, since Ia-like antigens continue to be expressed on some hybrid cells, which have therefore retained at least one characteristic surface marker from their parental B cell.

II. "Myeloid" Antigens

These antigens were expressed by HL-60 cells, but not by the B-cell line P_3HR-1, while K562 cells only showed reactivity with the antibodies TÜ8 and TÜ9. Although HP-1 hybrid cells seem to have lost all functional attributes of their myeloid parent HL-60 [4], they appeared to retain certain "myeloid" surface antigens, as shown in Table 3. A preliminary study of several clones from HP-1 cells (Zeuthen and Ziegler, unpublished) shows that it may be possible to obtain clones which do not bear most of the antigens characteristic for the myeloid cell types detected here. On the other hand, PUTKO1 cells appeared to have lost the ability to express the antigens detected by TÜ8 and TÜ9, although they are much more similar to K562 than to their other parent [3].

Since these antigens are glycosphingolipids (Towbin and Ziegler, unpublished), it may be of interest to examine the activity of glycosylases and glycosyltransferases in the hybrid cell lines employed here.

The results make it likely that gene dosage effects cannot be solely responsible for the observed phenomena. Furthermore, the phenotype of a hybrid cell cannot be predicted with certainty from the properties of the parental cells.

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