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Genetics

Expression of human myeloid-associated surface antigens in human–mouse myeloid cell hybrids

(chronic myeloid leukemia/Philadelphia chromosome/monoclonal antibodies/chromosome 11)

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ABSTRACT **Hybrid cell lines were obtained after fusion of mouse myeloid cells (WEHI-TG) with leukocytes from two patients with chronic myeloid leukemia. A third fusion was carried out with leukocytes from a patient with acute lymphocytic leukemia. All three patients carried the Philadelphia chromosome (Ph^1) in the leukemia cell population. Cytochemical analysis confirmed the myelo-monocytic nature of the hybrid cell lines. The presence of Ph^1 translocation products could be established in most hybrids derived from the two chronic myeloid leukemic patients, which confirms that indeed human myeloid cells were fused. Several of these hybrid lines showed reactivity with monoclonal antibodies known to be specific for human myeloid cells, whereas interlineage Chinese hamster fibroblast–human chronic myeloid leukemia hybrids failed to react with these antibodies. Five independently obtained monoclonal antibodies—MI/N1, UJ-308, VIM-D5, FMC-10, and B4.3—showed very similar reactivity patterns when tested on the hybrid clones. This result substantiates the evidence obtained from other studies, that these five antibodies are directed against the same myeloid-associated antigen. The gene(s) for expression of the latter antigen could be assigned to human chromosome 11.**

Hybrid cell lines, derived from fusion of cells within the hemopoietic system, have been isolated by several investigators (1–7). In such hybrids the genetic control of expression of specific blood cell characteristics can, in principle, be studied. It has been shown that (at least) some of the differentiation programs of different types of blood cells are mutually exclusive (1, 2). Fusion of cells of a specific state of differentiation generally results in the expression of the differentiation-associated traits of both fusion partners in the hybrids. The generation of antibody-producing hybrids by Köhler and Milstein (3) is a well-known example of the latter. Recently, secretion of human Ig heavy and light chains in intralineage human–rodent cell hybrids has been found (4, 5); also, expression of the human α-globin gene (6) and the expression of the erythrocyte-rosette receptor (7) have been reported. So far, the production of intralineage human–rodent hybrid cell lines expressing human myeloid characteristics has not been published.

We report here the isolation of proliferating human myeloid–mouse myeloid cell hybrids, in which human chromosomes segregate and which express characteristics specific of human myeloid cells. A human myeloid-associated antigen, detected by five monoclonal antibodies, could be assigned to human chromosome 11, when using these hybrids.

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MATERIAL AND METHODS

Patient Material and Production of Hybrid Cell Lines. Leukocytes from three patients (S.P., D.Y., and F.) with chronic myeloid leukemia (CML) and one patient (R.O.) with acute lymphocytic leukemia (ALL) were used for the cell fusion experiments. When leukocytes were obtained, patient S.P. was in the terminal blastic phase of the disease, marked by additional karyotypic changes besides the Philadelphia (Ph^1) translocation: 48,XY,+8,t(9q+;22q−),i(17q),+22q−. Patients D.Y. and F. were in a chronic phase of the disease. F. carried the classical Ph^1 translocation, whereas D.Y. carried a complex Ph^1 translocation in the immature dividing leukocytes: 46,XX, t(1p−;9q+;22q−). The leukemia cells of patient R.O. carried the Ph^1 translocation as well: 46,XY,t(9q+;22q−).

From the *in vitro* established mouse myeloid cell line WEHI-3B (8), a hypoxanthine phosphoribosyltransferase-deficient ($HPRT^-$) mutant was obtained after UV irradiation (10 J/m^2), followed by culture in medium containing 10 μg of 6-thioguanine per ml. The drug-resistant line (WEHI-TG) failed to incorporate [^{3}H]hypoxanthine. Isolation of the thymidine kinase-deficient (TK^-) Chinese hamster fibroblast cell line a23 has been reported previously (9). Fusions between patient-derived leukocytes and WEHI-TG cells were carried out according to standard procedures. Inactivated Sendai virus was the fusogen and hybrid selection was carried out in hypoxanthine/aminopterin/thymidine (10) medium. After fusion, cells were either seeded in methylcellulose-supplemented (1.2%) medium in dishes with a 0.5% agar base or in T30 flasks (Falcon) without the addition of methylcellulose or agar. Subcloning experiments were carried out in methylcellulose-supplemented cultures. The isolation of hybrids derived from fusion of Chinese hamster fibroblasts (a23) with CML cells has been described earlier: 9CB hybrids were obtained by using leukocytes from patient F. (11), whereas for the isolation of the 12CB hybrids leukocytes from patient D.Y. were used again (12). Cells were grown in F10 or RPMI 1640 medium with 10–15% fetal calf serum/2 mM glutamine/penicillin at 100 units/ml/streptomycin at 100 μg/ml.

Immunoassays and Antisera. The indirect immunofluorescence test described by Verheugt *et al.* (13) was used throughout this study, whereas the immunoperoxidase technique described by Mason *et al.* (14) was used to demonstrate the presence of intracytoplasmic antigen. Immunoprecipitations were carried out as described by Borst *et al.* (15), after ^{125}I labeling, according to Fraker's method (16). The precipitates were ana-

Abbreviations: CML, chronic myeloid leukemia; ALL, acute lymphocytic leukemia; HPRT, hypoxanthine phosphoribosyltransferase; TK, thymidine kinase.

lyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis in 10% acrylamide slab gels (17).

Monoclonal antibody MI/N1 was raised against neuroblastoma cells (18) and monoclonal antibody UJ-308 was raised against human fetal brain cells (J. T. Kemshead, personal communication). B4.3 and B13.9 are monoclonal antibodies obtained after immunization of a mouse with peripheral human leukocytes (19) and monoclonal antibody VIM-D5 was raised against human cell line K562 (20). Immunization of mice with human granulocytes yielded monoclonal antibodies FMC-10, -11, -12, and -13 (21). Within the hemopoietic system, all of these monoclonal antibodies react specifically with myeloid cells. Conventional antisera were used to detect the c-ALL antigen (22) and the Ia antigen (anti-SB), whereas monoclonal antibody 3A1 (23) was used to detect T-cell determinants.

Chromosome and Isoenzyme Analysis. Air-dried chromosome spreads were R-banded with acridine orange after heat denaturation. At least 16 metaphases of each hybrid line were analyzed. Lactate dehydrogenase (EC 1.1.1.27) isoenzymes were assayed by cellulose acetate gel (Cellogel) electrophoresis (24). The same populations of cells were used for immunologic, chromosome, and isoenzyme analyses.

Cytochemistry. Cytospin preparations were assayed for α-naphthyl acetate esterase, Sudan black, naphthol AS-D chloroacetate esterase, acid phosphatase, and periodic acid–Schiff by using standard procedures (25).

Table 1. Presence or absence of human leukemia-associated chromosomal abnormalities and five cytochemical reactions in WEHI-TG parental cells and 31 derived hybrid cell lines

Cells	Human leukemia-associated chromosome markers	Cytochemical reactions				
		SB	NCAE	α-NAE	AP	PAS
WEHI-TG		+	−	+	−	−
WESP-1	22q−	(+)	+	(+)	−	+ (10%)
WESP-2	22q−; i(17q)	ND	ND	ND	ND	ND
WESP-5	22q−; i(17q)	−	+	(+)	+	−
WESP-6	22q−	(+)	+	(+)	−	+
WESP-11	22q−	(+)	+	(+)	−	+ (<10%)
WEDY-1	1p−; 22q−	−	−	+	−	−
WEDY-3	—	(+)	−	+	−	+
WEDY-5	—	−	−	+	−	+
WEDY-7	9q+	+	−	+	S+	−
WEDY-8	22q−	ND	ND	ND	ND	ND
WEDY-9	22q−	(+)	−	+	ND	+ (<10%)
WEDY-10	1p−; 9q+; 22q−	+	S+	+	S+	+
WEDY-11	1p−; 22q−	−	−	+	−	+ (<10%)
WEDY-12	9q+	−	−	+	−	+
WEDY-13	1p−; 22q−	+	+	+	−	−
WEDY-14	22q−	+	−	+	−	+ (<10%)
WEDY-15	1p−; 22q−	+	+	+	−	+
WEDY-16	22q−	+	−	+	−	−
WEDY-17	22q−	−	−	+	−	+
WEDY-18	1p−; 22q−	−	−	+	−	S+
WERO-1	—	+	−	+	S+	+
WERO-2	—	+	+	+	−	+
WERO-3	—	+	+	+	S+	+
WERO-4	—	(+)	−	+	S+	S+
WERO-5	—	+	−	+	S+	+
WERO-6	—	ND	ND	ND	ND	ND
WERO-8	—	+	S+	+	+	+
WERO-9	—	+	−	+	+	+
WERO-10	—	+	+	+	−	+
WERO-11	—	+	−	+	+	S+
WERO-12	—	−	−	+	−	+

SB, Sudan black; NCAE, naphthol AS-D chloroacetate esterase; α-NAE, α-naphthyl acetate esterase; AP, acid phosphatase; and PAS, periodic acid–Schiff. (+), Weak expression. S+, <1% positive. ND, not determined.

RESULTS

Independent hybrid cell lines were obtained after fusion of WEHI-TG cells with leukocytes from S.P. (WESP), D.Y. (WEDY), and R.O. (WERO). These hybrid lines grow in suspension. Fusion of Chinese hamster a23 fibroblasts with leukocytes from F. and D.Y. provided the 9CB and 12CB hybrids, respectively. These hybrids grow in monolayer as do the parental a23 cells.

CML-associated aberrant human chromosomes were found in all but two WESP and WEDY clones (Table 1 and Fig. 1), indicating that human myeloid cells were fused. Ph^1 translocation-derived chromosomes were observed in the two 9CB and in five of seven 12CB hybrids as well (data not shown). In contrast, WERO hybrids lacked Ph^1 translocation products.

All WESP, WEDY, and WERO hybrids tested were positive (Table 1) for α-naphthyl acetate esterase, whereas several hybrid lines were positive for Sudan black or naphthol AS-D chloroacetate esterase, or both. These results confirm the myelomonocytic nature of these hybrids. In addition, some of the clones were positive for acid phosphatase or periodic acid–Schiff.

Immunologic characterization of the parental cells and the different hybrid lines is shown in Table 2. Monoclonal antibodies MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10 reacted with 95% of the peripheral leukocytes of patient D.Y. Per-

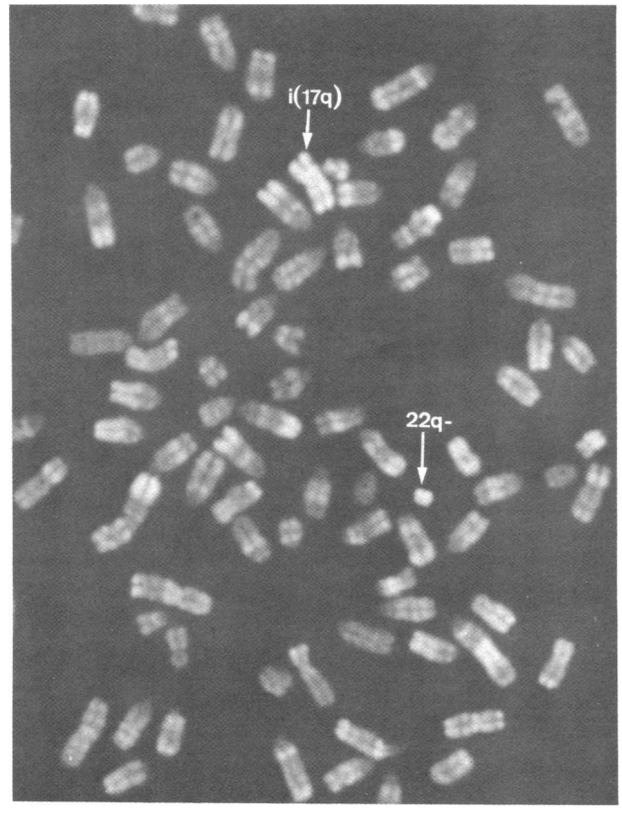

FIG. 1. Metaphase of hybrid cell line WESP-2 showing, in addition to the WEHI-TG mouse chromosomes, a variety of human chromosomes. The CML-specific Ph^1 chromosome (22q−) and i(17q) chromosome are indicated. The air-dried spread was heat-denatured and stained with acridine orange (R-banding).

Table 2. Reactivity patterns of 11 antibodies with parental cells and WEHI-TG or a23-derived hybrid cell lines

	Antibodies						
Cells	*	FMC-11	FMC-12	FMC-13	B13.9	c-ALL	3A1
a23	–	ND	ND	ND	–	–	ND
WEHI-TG	–	–	–	–	–	–	–
SP	ND	ND	ND	ND	ND	ND	ND
WESP-1	–	–	–	–	–	–	–
WESP-2	+ (30%)	ND	ND	ND	–	–	–
WESP-5	+ (50%)	+ (4%)	+ (8%)	+ (14%)	–	–	–
WESP-6	+ (10%)	+ (2%)	+ (4%)	–	–	–	–
WESP-11	–	–	–	–	–	–	–
DY	+ (95%)	+ (94%)	+ (89%)	+ (100%)	+ (83%)	–	–
WEDY-1	–	–	–	–	–	ND	–
WEDY-3	+ (30%)	–	+ (1%)	+ (2%)	–	–	–
WEDY-5	–	–	–	–	–	–	–
WEDY-7	–	–	–	–	–	ND	–
WEDY-8	–	–	–	–	–	–	–
WEDY-9	–	–	–	–	–	–	–
WEDY-10	+ (20%)	+ (6%)	+ (5%)	+ (15%)	–	–	–
WEDY-11	–	–	–	–	–	–	–
WEDY-12	–	–	–	–	–	–	–
WEDY-13	–	–	–	–	–	–	–
WEDY-14	–	–	–	–	–	–	–
WEDY-15	–	–	–	–	–	–	–
WEDY-16	–	–	–	–	–	–	ND
WEDY-17	–	–	–	–	–	–	–
WEDY-18	–	–	–	–	–	–	–
RO	+ (10%)†	ND	ND	ND	ND	+ (50%)	ND
WERO-1	+ (30%)	+ (1%)	+ (1%)	+ (4%)	–	–	–
WERO-2	+ (20%)	+ (8 %)	+ (5%)	+ (11%)	–	–	–
WERO-3	–	–	–	–	–	–	–
WERO-4	–	–	–	–	–	–	–
WERO-5	–	–	–	–	–	–	–
WERO-6	+ (15%)	+ (5%)	+ (3%)	+ (4%)	–	–	–
WERO-8	+ (30%)	+ (2%)	+ (2%)	+ (15%)	–	–	–
WERO-9	+ (70%)	+ (15%)	+ (15%)	+ (12%)	–	–	–
WERO-10	+ (50%)	+ (11%)	+ (13%)	+ (26%)	–	–	–
WERO-11	+ (45%)	+ (15%)	+ (15%)	+ (20%)	–	–	–
WERO-12	+ (30%)	+ (13%)	+ (6%)	+ (16%)	–	–	–
9CB-4+	–				–		–
9CB-14	–				–		–
12CB-4A	–				–		–
12CB-4B	–				–		–
12CB-14B	–				–		–
12CB-17B	–				–		–
12CB-20B	–				–		–
12CB-24D	–				–		–
12CB-27B	–				–		–

Identical results obtained with MI/N1, UJ-308, VIM-D5, FMC-10, and B4.3 are combined. Scores are based on indirect immunofluorescence tests. ND, not determined. +, In 9CB and 12CB series, FMC-11, -12, and -13 and c-ALL were not tested.

*MI/N1; UJ-308; VIM-D5; FMC-10; B4.3.

†Only B4.3 tested.

centages of FMC-11, -12, and -13 reactive cells ranged from 89% to 100%, whereas 83% were positive with B13.9. No c-ALL- or 3A1-positive cells were found. Only a minority (10%) of the bone marrow cells of patient R.O. in the ring fraction obtained by Ficoll-Isopaque centrifugation were positive with monoclonal antibody B4.3, whereas 50% of these blast cells were c-ALL antigen-positive, and 70% were Ia antigen-positive (data not shown). None of the antisera used reacted with WEHI-TG cells, dimethyl sulfoxide-stimulated WEHI-TG cells, or normal BALB/c granulocytes. Similarly, a23 cells failed to react with the human myeloid specific antisera. Cells of patients S.P. and F. were not tested for the presence of the antigens in question.

In the indirect immunofluorescence assay 13 hybrids reacted with monoclonal antibodies MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10, -11, -12, and -13 (Table 2). Identical patterns of reactivity were obtained with the former five antibodies, whereas

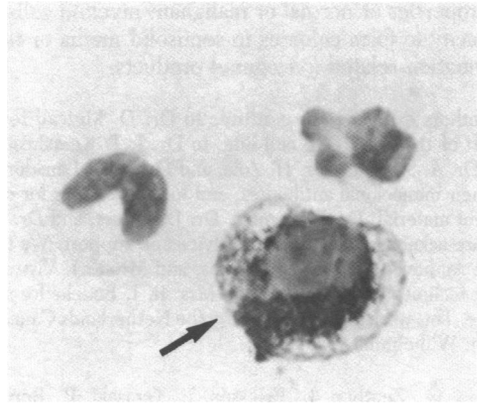

FIG. 2. Intracytoplasmic presence of human myeloid-associated antigen in a cell (arrow) of hybrid WESP-5. The antigen was visualized by using an immunoperoxidase technique and monoclonal antibody B4.3. Morphology of the nuclei is characteristic of myeloid cells.

for FMC-11, -12, and -13 lower frequencies of positive-reacting cells were found. The 9CB and 12CB hybrids did not react with MI/N1, UJ-308, VIM-D5, B4.3, or FMC-10. None of the hybrids tested was positive with B13.9, c-ALL, or 3A1. Presence of intracytoplasmic antigen was demonstrated in WESP-2 and WESP-5 with monoclonal antibodies B4.3 and FMC-10 by using an immunoperoxidase technique (Fig. 2). The latter was also observed in immature and mature human myeloid cells. The frequencies of positive-reacting hybrid cells were similar

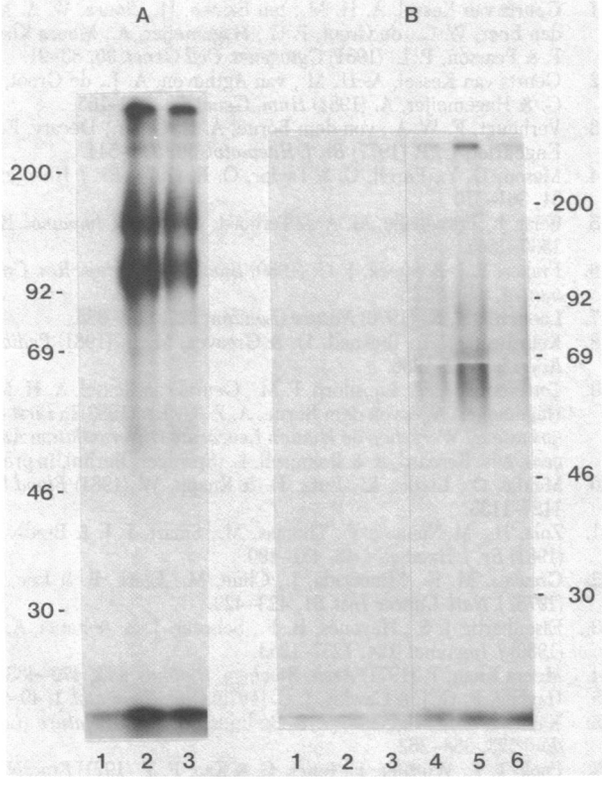

FIG. 3. (*A*) Immunoprecipitation patterns of human granulocytes with normal mouse serum (control) (lane 1), monoclonal antibody B4.3 (lane 2), and FMC-10 (lane 3). (*B*) Precipitation patterns of WEHI-TG cells with normal mouse serum (lane 1), B4.3 (lane 2), and FMC-10 (lane 3) and of hybrid cell line WESP-5 with B4.3 (lane 4), FMC-10 (lane 5), and normal mouse serum (lane 6). Two major bands with apparent M_rs of 105,000 and 150,000 are precipitated from granulocytes, whereas two major bands with apparent M_rs of 60,000 and 80,000 are precipitated from hybrid WESP-5. M_rs are shown as $M_r \times 10^{-3}$.

Table 3. Relationship between the human myeloid-associated antigen detected by MI/N1, UJ-308, VIM-D5, FMC-10, and B4.3 and human chromosomes in 64 primary and secondary hybrid cell lines

Chromosome	Chromosome/antigen, no. of clones +/+	+/−	−/+	−/−
1	11	27	4	22
2	13	20	2	29
3	11	9	4	40
4	13	19	2	30
5	7	19	8	30
6	11	42	4	7
7	14	24	1	25
8	12	20	3	29
9	6	2	9	47
10	14	40	1	9
11	13	0	2	49
12	14	17	1	32
13	6	13	9	36
14	9	24	6	25
15	6	7	9	42
16	12	16	3	33
17	5	17	10	32
18	4	16	11	33
19	11	19	4	30
20	10	30	5	19
21	15	34	0	15
22	13	12	2	37
X	12	45	3	4
Y	4	0	11	49
1p−*	0	7	15	42
9q+*	2	1	13	48
i(17q)*	4	4	11	45
22q−*	4	40	11	9

*Leukemia-associated chromosomal abnormalities.

to those obtained with the indirect immunofluorescence assay.

From the primary hybrid cell lines WESP-1, -2, -5, -6, and -11 and WEDY-1, 33 subclones were isolated. All of the 64 hybrid lines (primary and secondary) were tested with monoclonal antibodies MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10 and the same batches of cells were assayed for the human chromosome content (Table 3). A close correlation was observed between reactivity with the five monoclonal antibodies and human chromosome 11 only. Moreover, the percentages of positive cells in the indirect immunofluorescence tests correlated closely with the frequencies of chromosome 11 scored in these populations (data not shown). In two exceptional clones (WEDY-3 and WERO-2) an intact chromosome 11 could not be identified, whereas antibody-reactive cells were found. Screening of the total panel of 64 hybrids for the presence of the human chromosome 11 marker lactate dehydrogenase A gave 100% concordance. Also WEDY-3 and WERO-2 were positive for lactate dehydrogenase A. All human chromosomes were represented at least once among the a23 × CML hybrid cell lines. Three of these lines contained human chromosome 11 and, in addition, Ph^1 translocated products, but they failed to react with MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10.

Immunoprecipitation (Fig. 3) of the antigens detected by monoclonal antibodies B4.3 and FMC-10 on the surface of hybrid cell lines WESP-2 and WESP-5 and the subsequent Na-$DodSO_4$/polyacrylamide gel electrophoretic analysis revealed two major bands with apparent M_rs of 60,000 and 80,000, whereas no such antigens were found on WEHI-TG cells. Similarly obtained immunoprecipitation patterns from normal human granulocytes revealed two bands with apparent M_rs of 105,000 and 150,000.

DISCUSSION

Proliferating myeloid hybrids were obtained after fusion of an established mouse myeloid cell line (WEHI-TG) with leukocytes from two Ph^1-positive CML patients and one Ph^1-positive ALL patient. Morphological, cytochemical, and immunological studies confirmed the myeloid character of the hybrids. In contrast to rodent fibroblast–human CML hybrids, they appeared to be suitable for the study of myeloid differentiation markers. Despite the fact that we failed to isolate Ph^1-positive hybrids in the fusion with ALL cells (WERO), our results seem to indicate that the mouse parental cell line determines the myeloid character of the hybrids.

Some clones reacted with monoclonal antibodies detecting human myeloid-associated antigens both on the surface and in the cytoplasm of the cells. Recently, we found by immunoprecipitation and competition binding experiments that monoclonal antibodies MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10 react with the same antigen present on human myeloid cells (19). This observation is supported by the identical reaction patterns exhibited by the hybrid panels tested in our present study. According to Zola *et al.* (21) monoclonal antibodies FMC-10, -11, -12 and -13 react with distinct surface antigens. This is also substantiated by the differences observed in frequencies of positive-reacting cells. None of the hybrids tested reacted with monoclonal antibody B13.9. This antibody is known to react with mature granulocytes only (19) and none of the hybrids showed complete morphologic maturation.

A close correlation was observed between reactivity of hybrid cells with monoclonal antibodies MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10 and the presence of chromosome 11, or its marker, lactate dehydrogenase A. This concordance suggests the provisional localization on chromosome 11 of one or more genes responsible for the expression of the human myeloid-associated antigen detected by these five antibodies. Although expressed in a much lower percentage of cells, FMC-11, -12, and -13 had similar segregation patterns, which suggests that chromosome 11 may also be involved in the expression of the antigens recognized by these antibodies. In the past, several antibodies have been described that appeared to be directed against human membrane determinants coded by genes located on chromosome 11 (26–30). These antigens appear to be different from the antigen detected by MI/N1, UJ-308, VIM-D5, B4.3, and FMC-10, because they show different tissue distributions. In contrast to the myeloid-associated antigen described here, several of these chromosome 11-encoded antigens are expressed in lymphocytes and erythrocytes and in human–rodent fibroblast hybrids. The antigen detected by monoclonal antibody F10.44.2 (30) is also expressed in human–mouse B-cell hybrids, whereas the myeloid-specific antibodies used in this study do not react with such hybrids (data not shown). Moreover, the antigen detected by monoclonal antibody W6/45 (29) differs in apparent molecular weight with the antigen studied here (M_r 16,000 versus M_rs 105,000 and 150,000).

The human chromosome 11-encoded antigen studied by Jones *et al.* (31) resides in a glycolipid, the biosynthesis of which requires participation of specific glycosyl transferases. Further characterization of the antigenic determinants recognized by the antibodies used in our study must be undertaken to determine whether glycolipids or carbohydrates, or both, are involved as well. Such human antigenic carbohydrate determinants may be present on mouse or mouse–human heteropolymeric carrier molecules in the hybrid cells, which, in turn, could explain the differences observed in immunoprecipitation patterns from hybrid cells and human granulocytes.

The hybrids reported here have shown to be useful for the chromosomal localization of myeloid-associated antigens. They may also be helpful in studying the genetic control over various other properties of normal or malignant myeloid cells, such as the capacity to form colonies in semisolid media or to express transformation-related (oncogene) products.

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