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Assignment of Gene for Human Cell-Surface Membrane Antigen Trop-4 to Chromosome 11

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Abstract—*The gene (named MFI6) for a surface membrane antigen, Trop-4, is assigned to human chromosome 11 on the basis of studies using a mouse monoclonal antibody, immunofluorescence, fluorescence-activated cell sorting (FACS), immunoprecipitation, and mouse–human lymphocyte hybrids. The Trop-4 antigen is present on all human cell lines tested, on peripheral blood monocytes and granulocytes, and on a small fraction of peripheral blood lymphocytes, but is absent from erythrocytes. The Trop-4 monoclonal antibody precipitates an 85,000-dalton glycopolypeptide from hybrid cells containing human chromosome 11. However, in a human cell line expressing this antigen, a larger-molecular-weight species, 100–105,000 daltons was coprecipitated with the 85,000-dalton glycopeptide, and under nonreducing conditions a larger compound of 110–125,000 daltons was obtained. Although the Trop-4 antigen is of similar molecular weight to the Mab-4 and F10.44.2 antigens previously assigned to chromosome 11, it is shown to be different from them.*

INTRODUCTION

With an increased understanding of the human genome, it is becoming evident that the genetic information often is arranged in large clusters of related genes. Such clusters have probably arisen by duplications of originally one primordial gene, gradually leading to the formation of clusters of as many as several hundred structurally and functionally related genes which then, through chromosomal rearrangements, have been spread out to different chromosomes. During evolution, such genes and gene clusters have then diversified both structurally and functionally. Several such gene families have been found, the immunoglobulin gene family being one of the largest and most well known (1). This family consists in itself of several related gene clusters located on different chromosomes, and furthermore shows a structural and evolutionary relationship with the MHC genes, β_2-microglobulin, Thy-1 antigen, and poly Ig receptor as well as with the genes for the T-cell receptor (2–7). All these genes code for integral or membrane-associated proteins, some of which, like the immunoglobulins, also are secreted. Another example of related (membrane) proteins are the transferrin receptor, p97 (a melanoma associated protein) and the soluble protein transferrin, all of which are capable of or involved in iron binding and transport (8, 9). These are coded for by genes on human chromosome 3 (10–12). In this example, the structural and functional relationship of these proteins suggests a

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genetic and evolutionary relationship, which is further strengthened by the assignment of the respective genes to the same chromosome.

With new techniques in somatic cell genetics, the human gene map is rapidly becoming more complete. Monoclonal antibodies have been especially useful in the study and assignment of genes for human surface-membrane antigens. Genes for such antigens have been assigned to different human chromosomes, and human chromosome 11 has been found to carry several such genes. In this paper, membrane antigen Trop-4, carried on a glycopolypeptide of 85–90,000 daltons, is assigned to human chromosome 11 and compared to two other similar chromosome 11-coded antigens Mab-4 and F10.44.2.

MATERIALS AND METHODS

Mouse–Human Lymphocyte Hybrids and Human Cell Lines Used and Culture of Cells. The set of lymphocyte hybrids used for this study was derived from a fusion of mouse AKR thymoma BW5147 cells ($HPRT^-$) with human concanavalin A-activated peripheral blood lymphocytes. The production of the hybrids and their chromosomal characterization have been described earlier (10, 13). These hybrids have been subcloned and used previously for assigning genes for cell-surface antigens identified by heteroantisera or monoclonal antibodies to chromosomes 3, 11, and 21 (10, 11, 14–16).

The continuous human cell lines tested for reactivity with the Trop-4 antibody were the T-ALL lines Molt-4, JM, and CCRF-CEM; the B-ALL line Ball-1; the non-T/non-B ALL line Nall-1; the EBV-transformed lymphoblastoid lines LBL-LCA, LBL-K, and LBL-E; the hairy cell leukemia line JOK-1; the erythroleukemia line K562; and the (pre)myeloid–monocytoid lines KG-1, ML-2, HL-60, and U-937. All cell lines were kindly provided by Dr. Leif Andersson, Department of Pathology, University of Helsinki.

Mouse–human hybrid clones, the mouse parental cell line BW5147, and the human cell lines were all grown in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco) in an atmosphere of 5% CO_2–95% air. All cultures were grown in presence of penicillin and streptomycin (100 units/ml).

Trop-4 Antibody. The production and initial characterization of the monoclonal antibody Trop-4 have been described previously (17). Mouse monoclonal antibodies were produced against a human choriocarcinoma cell line BeWo. The antibody designated Trop-4 identifies an antigen present on human chorion carcinoma cell lines tested (BeWo, JEG), on fibrosarcoma (HT1080C), cervix carcinoma (HeLa 39), erythroleukemia (K562), and lymphoid (Ramos B-lymphoma and Molt-4) cell lines as well as on peripheral blood mononuclear cells but not on platelets or on erythrocytes. The antibody from subclone 162-28.2 used in this study was purified on DEAE–Sephacel, is an IgG2a isotype, and contains 2.2 mg protein/ml.

Immunofluorescence Analysis and FACS. The reactivity of the Trop-4 antibody with mouse–human hybrid clones was determined by indirect immunofluorescence and a FACS IV cell sorter. The staining was done as a two-step procedure by using the monoclonal antibody Trop-4 as the first step and an affinity chromatography-purified, fluorescein-conjugated, rabbit anti-mouse IgG antibody (Tago, Burlingame, California) as the second-step reagent. The human erythroleukemia line K562 expressing the Trop-4 antigen was used as a positive control. The mouse parental cell line BW5147 and a sample of each hybrid clone tested (with the second-step antibody alone) were used as negative controls.

Seven mouse–human hybrid clones were screened for reactivity with the Trop-4 antibody and a week later, two of these were reanalyzed and sorted into Trop-4-positive

and -negative fractions. After six days of culture, the positive and negative fractions were reanalyzed with the cell sorter.

The reactivity of the antibody with continuous human cell lines was analyzed with the cell sorter using a FITC-conjugated F $(ab')_2$ fragment of goat IgG against mouse IgG (Cappel, Cochranville, Philadelphia, Pennsylvania) as the second-step reagent.

Biochemical Characterization of Trop-4 Antigen. Cells of the human myeloid cell line U-937 were labeled using the periodate–$[^3H]NaBH_4$ labeling method (18), lysed in 1% Triton X-100, and used for immunoprecipitation as described previously (14, 19).

Polyacrylamide slab gel electrophoresis of the radioactive antigens was performed in the presence of sodium dodecyl sulfate according to Laemmli (20) using an acrylamide concentration of 8%. When indicated, the 2-mercaptoethanol was omitted. The gels were treated for fluorography as described (21). ^{14}C-labeled marker proteins were obtained from Amersham Int. Ltd. (Amersham, U.K.).

SDS-PAGE analysis of immunoprecipitates revealed higher-molecular-weight components under nonreducing conditions. To define these, immunoprecipitates were electrophoresed in cylindrical gels under nonreducing conditions. The gels were then sliced and the radioactive antigens eluted in 0.1% SDS and reanalyzed under reducing conditions on SDS slab gels. For control, duplicate samples were run under nonreducing conditions.

In order to compare the antigen precipitated by Trop-4 antibody with those identified by the monoclonal antibodies Mab-4 (14) and F10.44.2 (22, 23), serial precipitations with Trop-4 and Mab-4 antibodies of radioactive surface antigens from U937 cells were carried out. After three serial precipitations with these antibodies, the lysates depleted of the Trop-4 antigen were used for cross-precipitation with the Mab-4 or with the F10.44.2 antibody, and those depleted of the Mab-4 antigen for precipitation with the other two antibodies. The precipitates were analyzed by slab gel electrophoresis as described above. The F10.44.2 antibody was kindly provided by Drs. Peter Goodfellow and Alan Tunnacliffe, ICRF Laboratory for Human Molecular Genetics, London.

Mouse–human hybrid clones reactive and nonreactive with the Trop-4 antibody were also surface labeled, lysed in Triton X-100, and used for immunoprecipitation with the Trop-4 antibody. The precipitates were analyzed by SDS-PAGE as described above.

Chromosome Analysis. Chromosome preparations were made by conventional methods and stained by a modification of the trypsin–Giemsa banding method (24). Twenty cells were analyzed from each clone. Since the karyotype of such hybrids is not stable and as clones gradually lose human chromosomes with time in culture, the chromosome analysis was done simultaneously (within three days) with the immunofluorescence and immunoprecipitation studies.

RESULTS

Chromosomal Assignment. Initial screening of seven mouse–human hybrid clones by FACS analysis showed three of the clones to be positive for the Trop-4 antigen. These three clones contained several human chromosomes, but carried chromosome 11 as the only common human chromosome which also was absent from all the negative clones (Table 1). Two of the positive clones were reanalyzed and sorted into Trop-4-positive and -negative fractions. At the time of sorting, clone 28-1 contained 27% Trop-4-positive cells and clone 28-4 contained 59% positive cells. The clones carried chromosome 11 in about 25% and 55% of the cells, respectively, as determined by chromosome analysis of 20 cells. On reanalysis of the sorted fractions six

Table 1. Human Chromosomes Present in Mouse–Human Hybrid Clones at Times for Analysis and Sorting with FACS for Trop-4 Antigen Expression

Clone	Human chromosome															Reactivity with Trop-4 antibody (%)
	2	3	4	5	6	7	8	10	11	12	14	18	19	20	21	X	
Screening																	
7-1					85[a]												
13-13																5	
13-19					50											5	
28-1	40	55		65	90	55		50	30	5	55	35			55	60	29
28-2		35		40	60			65	60				20		30	60	74
28-4			20	55	75	60	20	45	65		50				50	25	65
28-5		40		35	45	20		20					20		70	45	
Sort																	
28-1	40	55		65	90	75		55	25	5	75	60			45	45	27
28-4		5	20	70	75	55	25	60	55		55				65	30	59
Reanalysis																	
28-1+	25	30		55	65	45		50	80	15	40	25		20	30	30	89
28-1−	10	20		40	60	15		15		5	35	15		15	40	50	
28-4+				35	70	40		30	85		55				25	25	93
28-4−				50	40	20		30			20				30	25	

[a]Percentage of cells carrying the chromosome of 20 cells studied.

days after sorting, the positive fractions of clones 28-1 and 28-4 had a purity of 89% and 93%, and carried the human 11 in about 80% and 85% of the cells, respectively. The negative fractions of either clone did not express the antigen and did not contain human chromosome 11 in any of the 20 cells studied. The sorting did not affect the retention of other human chromosomes in the different cell fractions. Thus, the expression of the Trop-4 antigen shows complete concordance with the presence of human chromosome 11 (Table 1).

Cell Distribution of Trop-4 Antigen. In the previous study, Trop-4 antigen was shown to be present on choriocarcinoma cell lines (BeWo, JEG), fibrosarcoma (HT1080C), cervix carcinoma (HeLa 39), erythroleukemia (K562), and lymphoid (Ramos, Molt-4) cell lines as well as on peripheral blood mononuclear cells but not on platelets or on erythrocytes. Thirteen additional hematopoietic cell lines of T-, B-, non-T/non-B, erythroid, or myeloid origin at different stages of differentiation were analyzed in this study and all were positive for the Trop-4 antigen (Table 2). However, only a smaller proportion (<50%) of Ficoll-Paque-isolated peripheral blood mononuclear cells were shown to express the antigen by FACS analysis, and by visual examination small lymphocytes were negative. Of fractionated T- and B-lymphocytes, less than 10% and 40%, respectively, were positive for the antigen. All monocytes and granulocytes, however, reacted with the Trop-4 antibody.

Biochemical Characterization of Antigen. Two major glycopolypeptides with apparent molecular weights of 85–90,000 and 100–105,000 were obtained from the U-937 human cell line by immunoprecipitation with Trop-4 antibody of radioactively labeled surface antigens followed by polyacrylamide gel electrophoretic analysis under reducing conditions (Fig. 1A). Under nonreducing conditions, a value of 110–125,000 was obtained for the larger molecular weight component coimmunoprecipitated with the peptide of 85–90,000 daltons (Fig. 1A). In order to identify the components of the higher molecular weight complex, nonreduced precipitates were electrophoresed in cylindrical gels, the gels

Table 2. Comparison of Reactivity of Four Monoclonal Antibodies Recognizing Chromosome 11-Coded Antigens with Human Cells by Indirect Immunofluorescence

Cell type	Reactivity (%)			
	Trop-4	Mab-4	F10.44.2	4F2
PBL	<50[a]	>90	+[b]	−[b]
T-cells	<10	85	+[b]	−[b]
B-cells	<40	65	(+)[b]	−[b]
Monocytes	+	+	+[b]	+[b]
Granulocytes	+	nd[c]	+[b]	−[b]
Red blood cells	−	−	+[b]	−[b]
JM	+	−	−	+
Molt-4	+	+	+	+
CCRF-CEM	+	+	nd	+[b]
Ball-1	+	+	+	+
LBL-LCA	+	+	nd	nd
LBL-K	+	+	nd	nd
LBL-E	+	+	nd	nd
JOK-1	+	(+)	nd	nd
Nall-1	+	+	+	+
K562	+	+	+	+
KG-1	+	(+)	nd	nd
ML-2	+	+	nd	nd
HL-60	+	(+)	nd	nd
RC-2A	+	+	nd	nd
U937	+	+	+	+

[a]On visual examination, small lymphocytes are negative by IDIF.
[b]Data based on literature (see ref. 29 and 30).
[c]nd, not done.

were sliced, and the radioactive antigens eluted from the gel slices. Duplicate samples of the eluted antigens were then analyzed on SDS slab gels under reducing conditions and under nonreducing conditions as control. In this way it was shown that the higher-molecular-weight component complex of 110–125,000 daltons contained the 85–90,000 component. The Mab-4 antigen also had an apparent molecular weight of 85–90,000 under reducing conditions, but a slightly lower molecular weight (80,000) under nonreducing conditions (not shown).

The Trop-4 antibody precipitated a single glycopolypeptide of 85–90,000 daltons from a mouse–human hybrid clone positive for the Trop-4 antigen by IDIF and carrying the human chromosome 11. The higher-molecular-weight component obtained from human cells was not immunoprecipitated from the hybrid clone. Neither of these peptides could be precipitated (Fig. 1B) from a clone carrying several other human chromosomes but not chromosome 11.

Comparison of Trop-4 Antigen with Mab-4 and F10.44.2 Antigens. In order to compare the Trop-4 and two other chromosome 11-coded antigens (Mab-4 and F10.44.2) which also are glycopolypeptides, and with similar molecular weights (85–90,000), serial immunoprecipitations were carried out with these three antibodies (see Table 3). Two parallel lysates of radioactive antigens depleted of the Trop-4 antigen by three sequential precipitations with this antibody were then used for precipitation, one with the Mab-4 antibody, and the other with the F10.44.2 antibody. Similarly, lysates depleted of the Mab-4 antigen were used for precipitation with the Trop-4 and F10.44.2 antibodies. In the first case, total depletion of the Trop-4 antigen from the lysate did not affect immunoprecipitation with the Mab-4 or with the F10.44.2 antibody (Fig. 1A, Table 3). In the parallel experiment, three serial precipitations

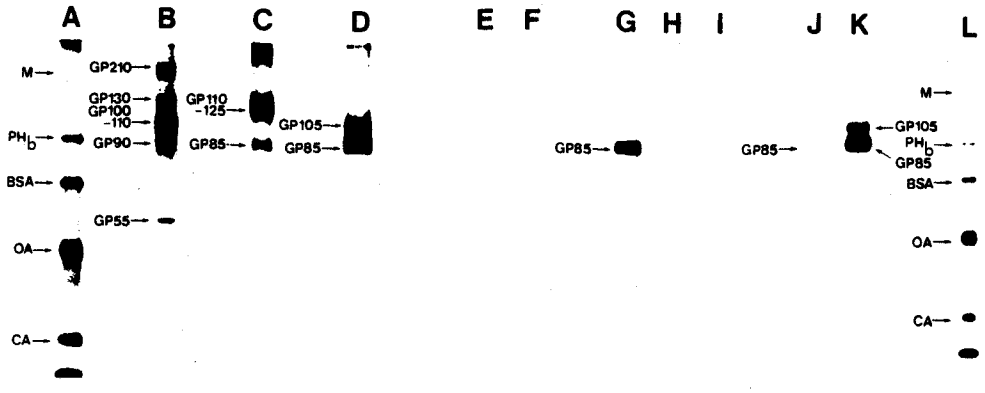

Fig. 1A. Polyacrylamide gel electrophoresis patterns of surface-labeled cells and immune precipitates obtained with monoclonal antibody Trop-4, and with monoclonal antibodies Mab-4 and F10.44.2 after preprecipitations with the Trop-4 antibody. (Lane A) ^{14}C-labeled molecular weight markers. M = myosin, PH_b = phosphorylase b, BSA = bovine serum albumin, OA = ovalbumin, CA = carbonic anhydrase. (B) Pattern of U-937 human cells. (C) Immune precipitate obtained with Trop-4 antibody from U-937 cells under nonreducing conditions. GP85 = surface glycoprotein with an apparent molecular weight of about 85,000. GP110–125 = surface glycoprotein with an apparent molecular weight between 110,000 and 125,000. (D) Immune precipitate obtained with Trop-4 antibody under reducing conditions, showing glycopolypeptides with apparent molecular weights of about 85,000 and 105,000. (E) and (F) Second and third serial precipitation with Trop-4 antibody from the same lysate as in (D). (G) Immune precipitate obtained with Mab-4 antibody after three serial preprecipitations with Trop-4 antibody (D–F). (H) and (I) Second and third serial precipitation with Trop-4 antibody (parallel experiment to D–G). (J) Immune precipitate obtained with F10.44.2 antibody after three serial preprecipitations with Trop-4 antibody. (K) Immune precipitate obtained with Trop-4 antibody as in (D). (L) ^{14}C-labeled molecular weight markers.

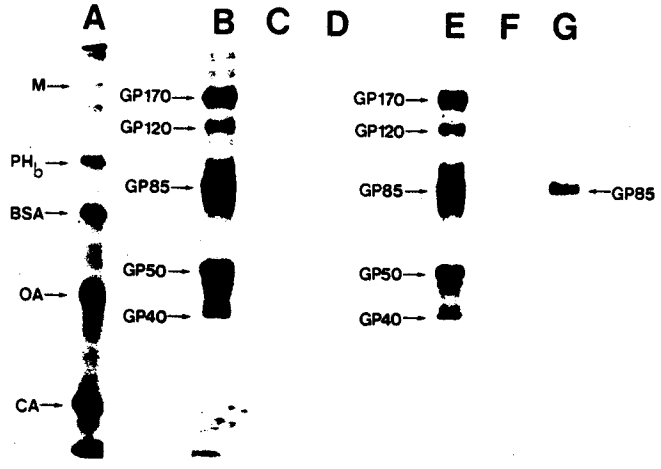

Fig. 1B. Polyacrylamide gel electrophoresis patterns of surface-labeled mouse–human lymphocyte hybrid cells and immune precipitates obtained with Trop-4 antibody and control serum. (Lane A) ^{14}C-labeled molecular weight markers as in Fig. 1A. (B) Pattern of hybrid clone carrying several human chromosomes but not the 11 and negative for the Trop-4 antibody. (C) Control precipitation from this hybrid clone with nonimmune mouse serum. (D) Immune precipitation from the same clone with Trop-4 antibody. (E) Pattern of hybrid clone carrying several human chromosomes as in (B) and also the human 11. (F) Control precipitation from this clone with nonimmune mouse serum. (G) Immune precipitate from the same clone obtained with Trop-4 antibody. The precipitates were analyzed under reducing conditions.

Table 3. Radioactivity Counts Precipitated by Serial Cross-precipitations with Trop-4, Mab-4, and F10.44.2 Antibodies

Precipitation	Antibody			
	Trop-4[a]	Trop-4[a]	Mab-4[a]	Mab-4[a]
1st precip.	1193[b]	1104	856	834
↓				
2nd precip.	224	196	566	626
↓				
3rd precip.	108	112	350	338
↓				
	786	502	608	308
Cross-precip. by antibody	Mab-4	F10.44.2	Trop-4	F10.44.2

[a]A Triton-X lysate of periodate–[^{3}H]$NaBH_4$-labeled surface antigens of human U-937 cells was divided in four identical samples for these four sets of parallel serial precipitations.
[b]CPM in 5 out of 200 μl of sample.

of radioactive lysates with the Mab-4 antibody diminished, but did not completely deplete, the lysates of the Mab-4 antigen, and a precipitate, even though much less, was still obtained with this antibody on the third precipitation. The F10.44.2 antibody precipitated approximately the same number of counts as the Mab-4 antibody and could be only weakly precipitated from the preprecipitated lysate (Table 3), while the Trop-4 antibody gave a strong precipitation.

DISCUSSION

In the mouse–human hybrid clones, the expression of the Trop-4 antigen showed complete concordance with the presence of human chromosome 11. In the hybrid clones tested for reactivity with the Trop-4 antibody by FACS analysis, all three clones carrying chromosome 11 expressed the antigen, and those lacking this chromosome were negative for the antigen. These results were further strengthened by sorting two heterogeneous clones into Trop-4-positive and -negative fractions, which showed a strong correlation to the presence/absence of human chromosome 11. The presence or absence of no other human chromosome correlated with the expression of the Trop-4 antigen.

As shown by immunoprecipitation and PAGE autoradiography analysis, in hybrid cells carrying the human chromosome 11, the Trop-4 antigen is expressed as a glycopolypeptide with an apparent molecular weight of 85–90,000. However, an additional glycopeptide of 100–105,000 (reduced conditions) or 110–125,000 (nonreduced conditions) daltons was coimmunoprecipitated by the monoclonal antibody to Trop-4 from human cells. The 100- to 105,000-dalton glycopeptide does not appear to be linked by disulfhide bonds to the 85,000-dalton peptide because the 85,000 peptide was precipitated under nonreducing conditions and also no complex corresponding to the combined molecular weight of the two peptides was seen. Reduction of the 110- to 125,000-dalton complex shows it to contain an 85- to 90,000-dalton glycopeptide, suggesting that the large component represents either a complex or that a processing change in the glycopeptide occurs in human cells. The observation that only the 85,000-dalton glycopeptide is expressed on hybrid cells carrying the human chromosome 11 is consistent with it being the Trop-4 antigen peptide.

The Trop-4 antigen was present on all the human cell lines tested of T-, B-, non-T/non-B, myeloid, or erythroid origin. When tested on peripheral blood cells, the antigen was present on monocytes and granulocytes, but only on a smaller fraction of peripheral

blood T- and B-lymphocytes. The antigen is not present on red blood cells. The expression of the Trop-4 antigen on peripheral blood cells differs clearly from that of the Mab-4 antigen and of the F10.44.2 antigen previously assigned to chromosome 11. These antigens are present on almost all peripheral blood lymphocytes, the F10.44.2 antigen being expressed on erythrocytes as well. The Trop-4 antigen is present on the JM cell line, whereas this line does not express the Mab-4 antigen.

The Mab-4 and F10.44.2 antigens are both carried on monomeric glycopolypeptides of approximately 85,000 daltons; the molecular weight for the first was previously reported as 75,000 daltons (14) and for the latter between 80,000 and 105,000 daltons (23). When run together on the same gel in a modified Laemmli system (under reduced conditions), all three antigens moved according to molecular weights of 85,000–90,000 daltons. However, as stated above, the Trop-4 antibody precipitated from human cells an additional, minor peptide of 100–105,000 daltons, but only the 85,000-dalton peptide was obtained from the mouse–human hybrid cells studied.

It could be argued on the basis of the molecular weights that all three antigens are carried on the same glycopolypeptide. This possibility is not in agreement with the cell distributions of the antigens and with their electrophoretic behavior under reducing/nonreducing conditions. It is definitely ruled out by the serial cross-precipitations with the three antibodies, which clearly show that the Trop-4 antigen is antigenically different from either the Mab-4 or the F10.44.2 antigen and is present on a separate membrane glycopolypeptide. However, the Mab-4 and F10.44.2 antibodies could recognize the same glycopolypeptide based on similarities in their reactivity both with different cell types and cellular antigens. Thus, in summary, the present study shows that the Trop-4 antigen is clearly different from the Mab-4 and the F10.44.2 antigens, even though all are of the same molecular weight and are coded for by genes on the same chromosome.

Genes for other membrane antigens with closely similar molecular characteristics also have been assigned to chromosome 11. These include the genes named MDU2 and MDU3, coding for peptides of 80,000 daltons, which are recognized by monoclonal antibodies A1G3 and A3D8 (25) and which may be similar to the F10.44.2 antigen (26), and the gene MDU1 coding for an antigen identified as a complex of the 80/40,000-dalton peptides (26), recognized by monoclonal antibodies 4F2 (25, 27) and TRA1.10 (28). The latter two antibodies recognize the 80,000-dalton peptide of the 80/40,000-dalton complex. Both peptides can be precipitated from human cells as well as from hybrid cells carrying the human 11. Whether the 40,000-dalton component is of mouse or human origin has not been determined (27). In addition to these molecular differences, the Trop-4 antigen differs from the 4F2/TRA1.10 antigen in its cell distribution: the Trop-4 antigen is present on a small fraction of peripheral blood lymphocytes while the 4F2/TRA1.10 antigen is absent from nonactivated peripheral blood lymphocytes (27, 28). Thus it would appear that Trop-4 is a new surface membrane antigen assigned to chromosome 11, and the gene is named MFI6 in accordance with international nomenclature rules. Whether a genetic relationship exists between the Trop-4 antigen and any other of these membrane antigens awaits further study.

The assignment of an additional gene for a cell-surface antigen to human chromosome 11 emphasizes the importance of this chromosome for human surface-membrane antigens. Most of the previous assignments have been made using either conventional antisera or monoclonal antibodies, and most of these antigens are still poorly characterized. In some cases the antigens may turn out to be identical, revealing only one gene mapped by different antibodies, as recently indicated by Jones et al. (26). In other cases the antigens

may prove to be related even though they are carried on different molecules (glycopeptides, glycolipids). This could be the case for carbohydrate antigens, when the actual gene mapped would code for a glycosyltransferase and not for the membrane protein. However, the accumulation of genetic information for human surface-membrane antigens to this chromosome is raising a question of possible genetic and evolutionary relationship of these, so far, both structurally and functionally poorly characterized membrane antigens.

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