GENETIC AND BIOCHEMICAL CHARACTERIZATION OF HUMAN LYMPHOCYTE CELL SURFACE ANTIGENS

The A-1A5 and A-3A4 Determinants

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Human < rodent interspecies hybrids express cell surface molecules derived from both parent species and thus have been useful in genetic analysis of the human cell surface (1, 2). The expressed human antigens serve as genetic markers that are powerful tools in both the analysis and manipulation of the human partial genome present in hybrid cells (2–5), and have recently allowed the development of a new technique for genetic mapping of cell surface antigens that combines somatic cell genetics and fluorescence-activated cell sorting (6, 7). However, the mapping of differentiation-specific surface markers has been hampered by difficulty in the construction of interspecies hybrids that retain the differentiated phenotype.

The monoclonal antibody A-1A5 recognizes a cell surface determinant expressed on all tested lymphoid cell lines. It is present at high levels on activated T cells but is only weakly expressed on resting lymphocytes. Nonactivated lymphocytes express a single A-1A5-specified component of 130,000 M_r as determined by immunoprecipitation. Several days after activation with mitogen or alloantigen, however, two additional subunit components of 165,000 and 210,000 M_r are coprecipitated by A-1A5 along with the 130,000 M_r protein (8). The appearance of these higher M_r subunits upon T cell activation occurs after the expression of early activation antigens, such as 4F2, the transferrin receptor, and the interleukin 2 (IL-2)[1] receptor (9), and thus represent late activation antigens. The component of highest molecular weight (210,000 M_r) appears to be recognized by the monoclonal antibody TS2/7, and thus is immunochemically distinct from the 130,000 M_r protein recognized by A-1A5 (10). Further evidence is required to demonstrate that the component of intermediate molecular weight (165,000 M_r) does not represent a modified form of the 130,000 M_r protein.

We have recently described (11) the development of a new class of interspecies

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[1] *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; IL-2 interleukin 2; NP-40, Nonidet P-40; PHA, phytohemagglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

hybrids that retain expression of differentiation-specific surface antigens during continued cell culturing. Using a panel composed of these hybrid lines, in conjunction with selection by the fluorescence-activated cell sorter (FACS), we have been able to map the genes for two antigenic markers that were previously inaccessible to genetic examination. The A-1A5 gene has been mapped to human chromosome 10 and progress has been made in determining the genetic relationship between the three A-1A5-defined subunits. Furthermore, we have mapped a determinant restricted to expression on hematopoietic cells and identified by monoclonal antibody A-3A4 to chromosome 4. This assignment represents the first cell surface marker mapped to that chromosome.

Materials and Methods

Tissue Culture. All tissue culture lines used in this study have a lymphocyte morphology and grow in suspension. All unhybridized cell lines were maintained without antibiotics in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY). The production and maintenance of these human < mouse lymphocyte hybrid lines that both continue to express lymphocyte-specific cell surface antigens and which proliferate in tissue culture have been described (11). These lines were maintained in selection medium consisting of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, HAT (hypoxanthine, 10^{-4} M; aminopterin, 5×10^{-7} M; thymidine, 10^{-4} M), ouabain (10^{-5} M), and antibiotics. In some cases, medium was further supplemented with five half-maximal units of rat T cell growth factor (Collaborative Research Inc., Waltham, MA). The human < mouse hybrids FRY-P1B1 (Frank Ruddle Yale-P1B1), FRY-P1B7, FRY-P1B8, and FRY-P1B9 were derived from a PEG 1540 (J. T. Baker Chemical Co., Phillipsburg, NJ)-induced fusion of phytohemagglutinin (PHA)-pretreated human peripheral blood lymphocytes enriched for T cells and the mouse AKR T lymphoma line BW5147 (11). The human < mouse hybrid lines FRY-M3H8, FRY-M2B12, FRY-M3H2, FRY-M3D2, FRY-M3A5, FRY-M3F2, and FRY-M2E10 were derived from a PEG 1540-induced fusion of the human leukemic T cell line MOLT-4 and BW5147 (11).

Monoclonal Antibodies and Antigen Immunoprecipitation. The monoclonal antibody A-1A5 has been characterized (8). The monoclonal antibody A-3A4 was derived from the same fusion as A-1A5 using previously described techniques (8). The monoclonal antibody TS2/7 immunoprecipitates a subset (210,000 and 130,000 M_r) of the proteins precipitated by A-1A5 (10). Cells from FACS-generated antigenically homogeneous hybrid cell lines and hybrid parental lines were labeled with ^{125}I. Membrane glycoproteins were extracted with Nonidet P-40 (NP-40), immunoprecipitated with the appropriate antibody, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (12).

Karyotype and Isozyme Analyses. Chromosome preparations were produced by standard methods that allowed genetic analysis of individual cells. Metaphase chromosome spreads were trypsin banded, treated with Giemsa stain according to the method of Wang and Fedoroff (13), and analyzed and photographed using a 63× oil immersion Zeiss objective with transmitted light. Isozyme analyses of hybrid populations were carried out using the cell preparations and starch gel electrophoresis procedures of Nichols and Ruddle (14) and Harris and Hopkinson (15), or the cellogel techniques of P. Meera Khan (16). Most isozymes will not be detected if present in <10% of a cell population (14). Hybrid populations were expanded in culture to 4×10^7 cells, allowing karyotype, isozyme, and FACS analyses to be performed on identical populations. This is crucial to the analysis since these hybrid lines undergo continual chromosome segregation.

Indirect Immunofluorescence Assays and Sterile Cell Sorting. Fluorescence analysis, specificity controls, and cell sorting procedures were performed as previously described (17). Control supernatants were used to provide a measure of both autofluorescence and nonspecific background fluorescence in experimental cell populations. Cells stained by

indirect immunofluorescence were initially examined for viability using the trypan blue dye exclusion test. Nonviable cells rarely exceeded 2% of the population. To ensure sterility in cell sorting, all tubing was extensively washed with 7X detergent (Flow Laboratories, Inc., McLean, VA) followed by 70% ethanol. Sorted cells were collected in 35 × 10-mm petri dishes containing RPMI 1640 tissue culture selection medium supplemented with garamycin (Schering Corp., Kenilworth, NJ). Sorted populations were reanalyzed ~7–10 d after initial sorting procedures. Nomenclature for the sorted lines is Parental line.antigen.number of selections for (SR^+) or against (SL^-) antigen.

Results

Chromosome Mapping of the A-1A5 Determinant. 12 human < mouse interspecies hybrid populations were analyzed by indirect immunofluorescence for expression of the A-1A5 surface determinant. 8 of the 12 hybrid populations derived their human partial genome from the MOLT-4 leukemic parent line, while the other 4 received their human chromosomes from normal peripheral T lymphocytes. Varying percentages of A-1A5-positive cells were observed both microscopically and by the FACS in four MOLT-4-derived lines and in three hybrid lines derived from normal T cells (Table I).

The heterogeneous population FRY-M3H8 was sorted with respect to expression of the A-1A5 determinant, producing sublines homogeneously positive and homogeneously negative for antigen expression. These lines were expanded in parallel and resorted until a maximum level of antigen homogeneity was attained. The A-1A5-positive subpopulation reached a maximum level of 88% homogeneity, while the negative subpopulation reached a level of 92% homogeneity (Fig. 1). These sorted tissue culture lines were designated FRY-M3H8.A-1A5.$SL3^-$ and FRY-M3H8.A-1A5.$SR8^+$, indicating that a total of three sorting procedures were required to generate the negative subpopulation whereas eight sorts generated the antigen-positive subpopulation (Fig. 1).

TABLE I

A-1A5 Antigen Gene Mapping

Cell lines	Percent A-1A5-positive cells	Human chromosomes																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
FRY-M3A5	0					X		X					X	X							X	X		X
FRY-M3F2	0	X	X		X		X					X				X	X		X			X		X
FRY-M3H2	27			X	X			X			X		X	X	X	X	X		X	X	X	X		X
FRY-M3D2	32	X									X					X			X					X
FRY-M2B12	36	X	X		X	X	X				X	X					X		X					X
FRY-P1B7	12		X				X			X	X			X		X		X	X			X		X
FRY-P1B8	18		X		X		X		X	X	X	X	X			X			X	X		X		X
FRY-P1B9	26						X		X	X	X		X	X		X			X			X	X	X
FRY-M3H8 unsorted	62				X				X		X				X	X			X					X
FRY-M3H8 A-1A5 3^-	8*		X												X	X			X					X
FRY-M3H8 A-1A5 8^+	88										X				X	X			X					X

Isozyme analysis of a series of human < mouse interspecies lymphocyte hybrid populations and FACS-derived, antigenically homogeneous subpopulations. The percentage of cells in the hybrid population expressing the A-1A5 antigen is presented in parentheses after the cell line. Cell preparations, starch gel electrophoresis, and cellogel techniques were performed as described in Materials and Methods. Cell sorter derivation of homogeneous sublines is described in the text. The presence of a human chromosome as defined by isozyme expression is indicated by an X. Boxes indicate that human chromosome 10 is the only chromosome fitting the required pattern.

* The isozymes adenosine kinase (2.7.1.20) and glutamic-oxaloacetic transaminase (2.6.1.1), used for the detection of human chromosome 10, are not sufficiently sensitive to detect subpopulations of <10% (see Table II).

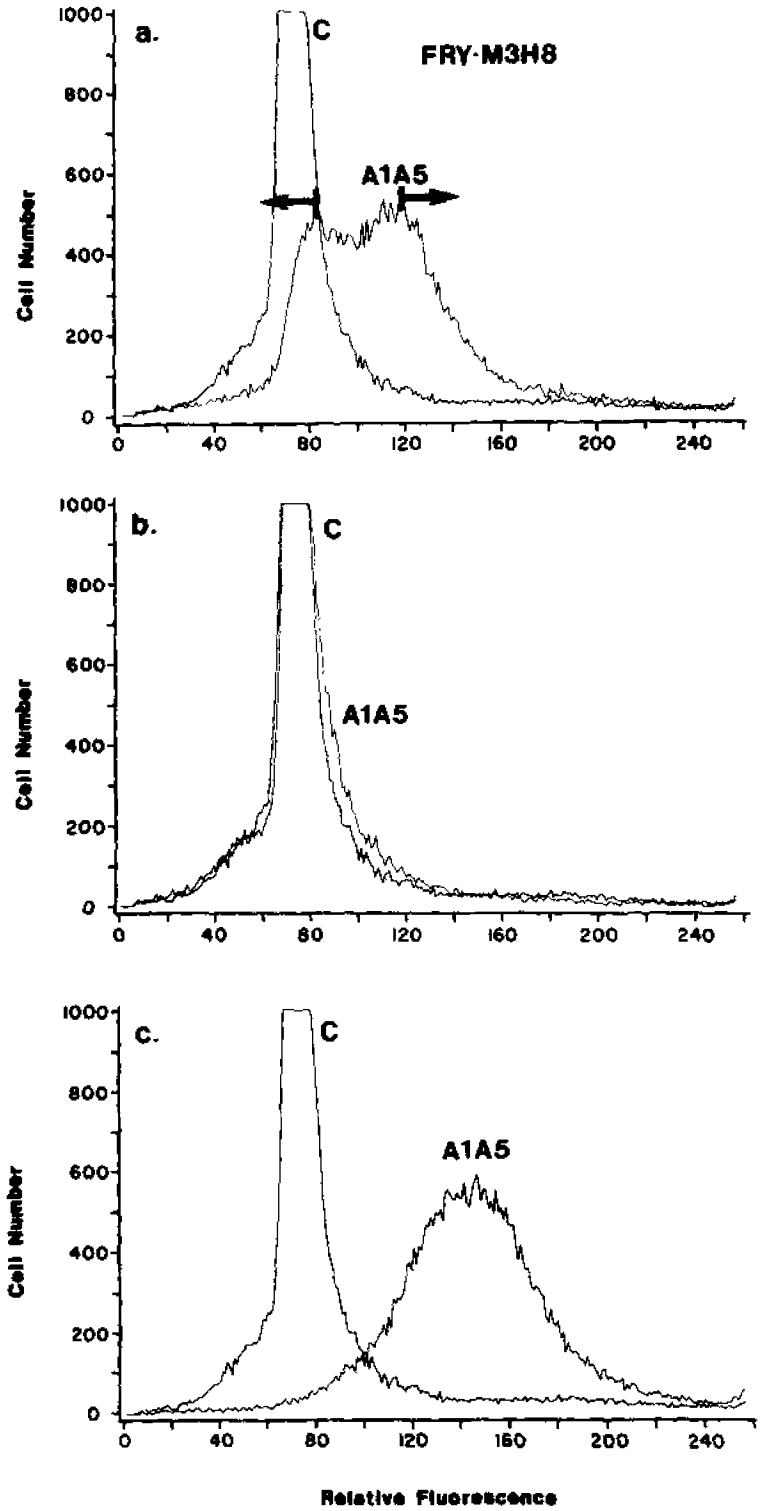

FIGURE 1. Analysis and cell sorting of human < mouse hybrid line FRY-M3H8 for expression of the A-1A5 antigen. Cells were stained by indirect immunofluorescence using monoclonal antibody A-1A5 or control supernatants (C) and analyzed using the FACS. Photomultiplier voltages were converted by logarithmic amplification for display on the multichannel analyzer. Cell sorting criteria are indicated by arrows. (*a*) FRY-M3H8, (*b*) FRY-M3H8.A-1A5.SL3$^-$, (*c*) FRY-M3H8.A-1A5.SR8$^+$.

Nine lymphoid-positive hybrid lines and the FACS-generated FRY-M3H8 sublines were analyzed for the presence of human isozymes to determine the identity of retained human chromosomes. Isozyme analyses indicated that all human chromosomes, except Y, were present in one or more of the cell populations, and that each line examined contained a unique subset of the human genome (Table I). Analysis of the FACS-generated homogeneous subpopulations and the unsorted parental hybrid cell lines shows that only chromosome 10 segregated concordantly with expression of the A-1A5 determinant (Table I).

Karyotype data for the A-1A5-positive and A-1A5-negative FACS-derived sublines are presented in Table II. Thirty-two individual chromosome spreads were analyzed for the A-1A5-positive population while 30 chromosome spreads were analyzed for the A-1A5-negative population. Human chromosome 10 was detected in 60 and 6.3% of the cells in these populations, respectively. The low percentage retention of chromosome 10 in hybrid FRY-M3H8.A-1A5.SL3$^-$ corresponds to the low percentage of A-1A5-positive cells detected in this population. Chromosome 10 was not detected in this hybrid cell population by isozyme analysis because of its low frequency representation in the population (Table I). Karyotypic analysis of the MOLT-4 human parent cell line revealed

TABLE II

Chromosomes Retained by Homogeneous Hybrid Subpopulations

Human chromosomes	Percent present	
	FRY-M3H8.A1A5.SL3$^-$	FRY-M3H8.A1A5.SR8$^+$
1	6.3	0
2	0	3.3
3	0	6.7
4	0	0
5	0	0
6	75.0	53.0
7	0	0
8	0	0
9	0	0
10	6.3	60.0
11	0	6.7
12	12.5	3.3
13	0	3.3
14	0	0
15	68.8	33.3
16	0	3.3
17	12.5	0
18	31.3	16.7
19	0	0
20	0	0
21	0	0
22	0	0
X	68.8	56.7

Human chromosomes retained by human < mouse hybrid cell populations as detected by karyotype analysis of Giemsa-banded hybrid cell genomes. 32 individual chromosome spreads were analyzed for the A-1A5-positive population, 30 for the A-1A5-negative population.

inversions involving chromosomes 2 and 5, and extra genetic material on the long arm of chromosome 7 (Human Genetic Mutant Cell Repository, IMR, Camden, NJ). None of these MOLT-4 marker chromosomes were observed in the 62 hybrid metaphase spreads analyzed.

Biochemistry of A-1A5 Antigen on Parental and Hybrid Lines. Immunoprecipitation of the A-1A5 antigenic determinant from the unhybridized human parent T cell leukemic line (MOLT-4) yielded subunits of 125,000 and 160,000 M_r (Fig. 2*g*). Similar results (subunits of 130,000 and 165,000 M_r) have been reported for the T cell leukemia line HSB (8). Both of these subunits were absent from the unhybridized mouse parent line BW5147 (Fig. 2*a*) and from the FACS-generated hybrid subpopulation FRY-M3H8.A-1A5.SL3$^-$ (Fig. 2*c*), neither of which express A-1A5. The A-1A5 antigen immunoprecipitated from the human < mouse hybrid line FRY-M3H8.A-1A5.SR8$^+$ consisted of only the 125,000 M_r subunit (Fig. 2*e*), indicating that the 160,000 M_r subunit was not present.

Provisional Chromosome Mapping of the TS2/7 Determinant. A monoclonal antibody specific for the 160–165,000 M_r protein present on MOLT-4 (see Fig. 2), as well as on activated T cells, has not yet been identified. Monoclonal antibody TS2/7 specifically recognizes the 210,000 M_r protein associated with the A-1A5

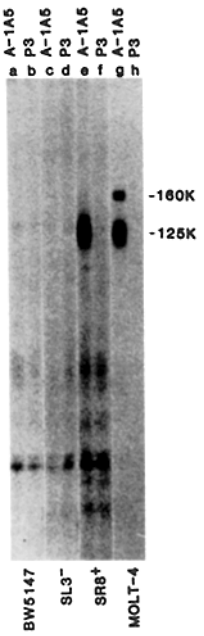

FIGURE 2. ^{125}I-labeled proteins immunoprecipitated by the monoclonal antibody A-1A5 from human < mouse somatic cell hybrid lines and from the human and mouse unhybridized parental cell lines. The mouse parental cell line (BW5147), human parental cell line (MOLT-4), FACS-generated subline negative for A-1A5 expression (SL3$^-$), and FACS-generated subline positive for A-1A5 expression (SR8$^+$) were labeled with ^{125}I, extracted with NP-40, and immunoprecipitated with either ~2 μg A-1A5 or the negative control, P3, as previously described (12). Gel analysis was on an 8% acrylamide gel in the presence of reducing agent. Standards myosin (200,000 M_r) and phosphorylase b (92,000 M_r) were used to estimate relative M_r of the immunoprecipitated proteins. Immunoprecipitates with A-1A5 are from BW5147 (*a*), SL3$^-$ (*c*), SR8$^+$ (*e*), and MOLT-4 (*g*). In control experiments the irrelevant antibody P3 was used with BW5147 (*b*), SL3$^-$ (*d*), SR8$^+$ (*f*), and MOLT-4 (*h*).

protein on activated T cells (10). Although this protein is not expressed on MOLT-4 cells (Fig. 2), FACS analysis demonstrated that some MOLT-4-derived hybrid cells did express the TS2/7 determinant (Table III). In contrast, TS2/7 binding was not observed on any hybrid line whose human parent was normal peripheral blood lymphocytes (results not shown). Comparison of TS2/7 reactivity with the pattern of individual chromosome retention as determined by isozyme analysis (Table III) suggests possible map positions for the TS2/7 locus on human chromosomes 10 or 15. The ratio of concordant/discordant segregation for this antigen and chromosome 10 or 15 is 7:1. A higher discordancy ratio is observed for all other chromosomes in this hybrid panel.

Since the determinant defined by the monoclonal antibody TS2/7 is closely associated with that defined by A-1A5, hybrid cell populations that had been sorted for A-1A5 homogeneity were examined for TS2/7 expression. No TS2/7 staining was present on the A-1A5-negative hybrid line FRY-M3H8.A-1A5.SL3$^-$ (partial karyotype, 10^-15^+) while hybrid line FRY-M3H8.A-1A5.SR8$^+$ (10^+15^+), which is 88% A-1A5-positive, displayed staining for the TS2/7 determinant on ~15% of the cells (data not shown). However, the TS2/7 antigen could not be detected after either TS2/7 (data not shown) or A-1A5 (Fig. 2) immunoprecipitation from the hybrid. All attempts to generate antigenically homogeneous hybrid cell populations with respect to the TS2/7 antigen were unsuccessful and resulted in uniform loss of TS2/7 reactivity.

Preliminary Characterization of the A-3A4 Determinant. After hybridoma production generated to human T cells, screening by immunoprecipitation revealed a monoclonal antibody, A-3A4, that recognized an ^{125}I-labeled protein (45,000 M_r) similar in size to the heavy chain of HLA-A,B,C antigens (Fig. 4*a*, *c*, *f*). However, unlike HLA, the A-3A4 antigen had no associated light chain. Long-term cytotoxic T lymphocyte lines, T and B lymphoblastoid cell lines, and the monocytoid line U-937 all expressed similar A-3A4-reactive proteins with no apparent size variation. Nonhematopoietic HeLa cells did not express A-3A4 (data not shown). The cell surface ^{125}I-labeled protein immunoprecipitated by A-3A4 had estimated M_r of 47,000, 46,000, and 42,000 on 10, 8, and 5–15%

TABLE III
TS2/7 Antigen Gene Mapping

Cell lines	TS2/7 reactivity	Human chromosomes																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
FRY-M3H2	+			X	X			X			X		X	X	X	X	X		X	X	X	X		X
FRY-M3D2	+	X									X					X			X					X
FRY-M4B4	−	X																	X					X
FRY-M3A5	−					X		X					X	X							X	X		X
FRY-M3F2	+	X	X		X		X					X				X	X		X			X		X
FRY-M2E10	−														X					X		X		X
FRY-M348 A-1A5 3$^-$	−		X												X	X			X					X
FRY-M3H8 A-1A5 8$^+$	+										X				X	X			X					X

Isozyme analyses of a series of human < mouse interspecies lymphocyte hybrid populations. The reactivity of cells in the hybrid population for the TS2/7 antigen is presented in parentheses after the cell line. Cell preparations, starch gel electrophoresis, and cellogel techniques were performed as described in Materials and Methods. The presence of a human chromosome as defined by isozyme expression is indicated by an X. Concordancy of antigen expression and chromosome retention allow provisional assignment of TS2/7 to chromosomes 10 or 15.

gradient gels, respectively. Thus, the A-3A4 antigen was assigned an average size of 45,000 M_r. In addition to ^{125}I-labeling, weak metabolic labeling with [^{35}S]methionine was observed, indicating that the A-3A4 antigen is biosynthesized rather than acquired or adsorbed from an exogenous source (Hemler, unpublished data).

Chromosome Mapping of the A-3A4 Determinant. 12 human < mouse hybrid lines (8 MOLT-4-derived lines and 4 normal peripheral T cell-derived lines) were examined by indirect immunofluorescence and FACS analysis for A-3A4 expression. Various percentages of positively stained cells were observed in three MOLT-4-derived hybrid lines and in two normal T cell-derived hybrid lines (Table IV).

The heterogeneous population FRY-P1B1 was sorted with respect to expression of the A-3A4-determinant, producing sublines homogeneously positive and homogeneously negative for antigen expression. These lines were expanded, reanalyzed, and resorted until maximal homogeneity was achieved. These sorted lines were designated FRY-P1B1.A-3A4.SL2$^-$ and FRY-P1B1.A-3A4.SR4$^+$ and were >99% and 86% homogeneous, respectively (Fig. 3).

The mouse parent line, the unsorted hybrid lines, and the sorter-generated sublines were analyzed for the presence of human isozymes to determine the identity of the human chromosomes retained. A comparison of the patterns of monoclonal antibody reactivity and of chromosome retention indicates that only human chromosome 4 segregates concordantly with A-3A4 reactivity (Table IV). Chromosome 18 is present in the antigen-expressing subline and absent in the subline not expressing antigen. However, it does not contain the antigen gene locus as evidenced by isozyme data from antigen-negative cell lines FRY-B1B8, FRY-1B7, and FRY-M3D2.

Immunoprecipitation of the A-3A4-reactive antigen on the hybrid line FRY-P1B1 is shown in Fig. 4*c*. The 45,000 M_r antigen from the hybrid cells appeared to be essentially the same as that derived from human peripheral blood lympho-

TABLE IV

A-3A4 Antigen Gene Mapping

Cell line	Percent A-3A4-positive cells	Human chromosomes																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
FRY-M3D2c	0	X									X					X			X					X
FRY-P1B7c	0		X				X			X	X			X		X		X	X			X		X
FRY-P1B9c	0						X		X	X	X		X	X		X			X			X	X	X
FRY-M3H2c	77			X	X			X			X		X	X	X	X	X		X	X	X	X		X
FRY-M3H8c	45				X				X	X					X	X			X					X
FRY-M2B12c	30	X	X		X	X	X				X	X					X		X					X
FRY-P1B8c	18		X		X		X		X	X	X					X			X	X		X		X
FRY-P1B1c	24	X	X		X		X			X				X	X	X			X	X			X	X
FRY-P1B1. A-3A4.SL2$^-$	0						X							X	X	X								X
FRY-P1B1. A-3A4.SR4$^+$	86				X		X							X	X	X			X					X

Isozyme analysis of a series of human < mouse interspecies lymphocyte hybrid populations and FACS-derived, antigenically homogeneous subpopulations. The percentage of cells in the hybrid population expressing the A-1A5 antigen is presented in parentheses after the cell line. Cell preparations, starch gel electrophoresis, and cellogel techniques were performed as described in Materials and Methods. Cell sorter derivation of homogeneous sublines is described in the text. The presence of a human chromosome as indicated by isozyme expression is indicated by an X. The chromosome containing the A-3A4 locus must be present in all antigen-positive populations and absent from all antigen-negative populations. Boxes indicate that human chromosome 4 is the only chromosome fitting the required pattern.

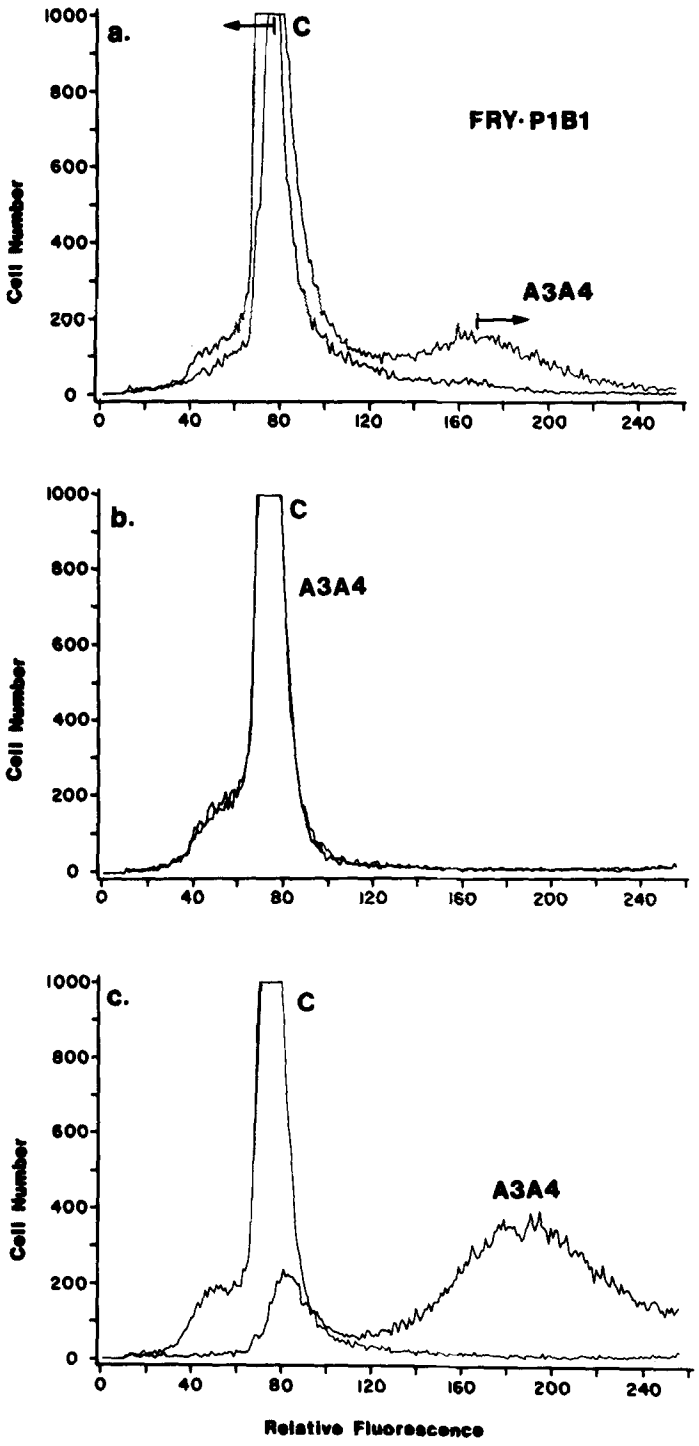

FIGURE 3. Analysis and cell sorting of human < mouse hybrid line FRY-P1B1 for expression of the A-3A4 antigen. Cells were stained by indirect immunofluorescence using monoclonal antibody A-3A4 or control supernatants (C) and analyzed using the FACS. Photomultiplier voltages were converted by logarithmic amplification for display on the multichannel analyzer. Cell sorting criteria are indicated by arrows. (*a*) FRY-P1B1, (*b*) FRY-P1B1.A-3A4.SL2$^-$, (*c*) FRY-P1B1.A-3A4.SR4$^+$.

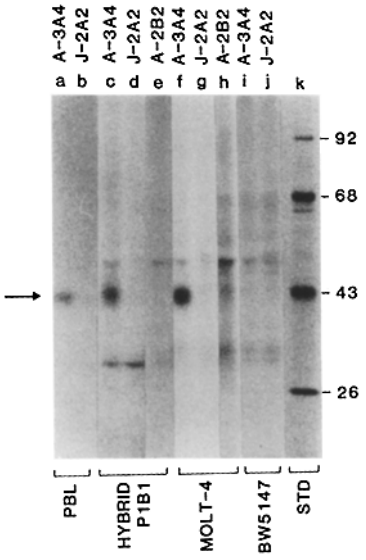

FIGURE 4. Immunoprecipitation of A-3A4-reactive, ^{125}I-labeled antigen from hybrids and unhybridized parent lines. Immunoprecipitates with A-3A4 are from human peripheral blood lymphocytes (PBL) (*a*) after 4-d stimulation with PHA (10 g/ml), the hybrid P1B1 (*c*), MOLT-4 (*f*), and the mouse parent BW5147 (*i*). The irrelevant antibodies J-2A2 (*b*, *d*, *g*, *j*) and A-2B2 (*e*, *h*) were used to show background proteins. Standards (*k*) were phosphorylase b (92,000 M_r), bovine serum albumin (68,000 M_r), ovalbumin (43,000 M_r), and chromotrypsin (26,000 M_r), and gel analysis was on a linear 5–15% acrylamide gel in presence of reducing agent.

cytes (Fig. 4*a*) or from the human lymphoblastoid cell line MOLT-4 (Fig. 4*f*). No A-3A4-reactive protein was immunoprecipitated from the mouse parent line BW5147. Control immunoprecipitations with the monoclonal antibodies J-2A2 and A-2B2 are included in Fig. 4 to indicate nonspecific background bands.

Discussion

Using the FACS, an antigenically heterogeneous hybrid cell population can be fractionated into two subpopulations, each of which is either homogeneously antigen positive or negative (6). The genomes of such paired homogeneous sublines should differ from each other only by the human chromosome or chromosomes responsible for expression of the target cell surface antigen. Using interspecies lymphocyte hybrid lines described previously (11), we have shown that the chromosomal map position of the gene coding for the A-1A5 antigenic determinant lies on human chromosome 10. Concordant segregation of A-1A5 with chromosome 10 was seen in hybrid lines derived from the human leukemic line MOLT-4, as well as in lines derived from human peripheral blood lymphocytes. Thus, the A-1A5 locus on chromosome 10 is unaffected by genomic rearrangements that may have occurred in the human leukemic parental cell line. Karyotype analysis of the A-1A5-positive and A-1A5-negative subpopulations ruled out the possibility of artifactual isozyme segregation caused by fragmentation of human chromosomes, and independently confirmed isozyme results by showing concordant segregation of A-1A5 expression with an intact human chromosome 10.

Using the interspecies lymphocyte hybrid lines described, it has also been demonstrated that the chromosomal map position of the gene coding for the A-3A4 determinant lies on human chromosome 4. As with the A-1A5 antigenic determinant, concordant segregation was demonstrated on hybrid lines whose human parent was the leukemic cell line MOLT-4 as well as on hybrid lines whose human parent was normal peripheral blood lymphocytes. This assignment represents the first cell surface marker to be mapped to human chromosome 4.

As with the hybrid cell populations sorted for homogeneous expression of the A-1A5 determinant, those sorted for A-3A4 expression failed to reach a level of 100% antigen homogeneity. Repeated selection by sorting failed to generate an expressing subpopulation of more than ~86% of the cell line. This supports the idea that the lymphocyte hybrids used in these mapping studies have a high rate of human chromosomal segregation in which even the short time elapsing between FACS analyses allows a substantial amount of chromosome segregation. In contrast, the low frequency appearance of chromosome 10 (Fig. 2) and A-1A5 (Table I) was observed in subsequent analysis of the antigen-negative sorts. This suggests that a subpopulation of hybrid cells containing chromosome 10 was A-1A5 negative during cell sorting but was able to reexpress the antigen due to the presence of the coding chromosome. Such antigenic modulation may be a function of cell cycle or growth rate variation. This was not observed during the analysis of the A-3A4 antigen.

A single-cell surface molecule termed EMP-130 (18) has previously been mapped to human chromosome 10. Although both the EMP-130 and the A-1A5 antigens are 130,000 M_r, EMP-130 has not been examined on cells of lymphoid lineage and the relationship between these molecules requires further study. Two T cell activation-related components (210,000 and 165,000 M_r) are associated with the 130,000 M_r A-1A5 antigen on activated T cells (8, 10). The component of highest M_r defined by the monoclonal antibody TS2/7 unexpectedly showed low level expression on MOLT-4 × BW5147 hybrids despite being unexpressed in the human MOLT-4 parent. This induction of expression by hybridization may be the result of a process paralleling T cell activation or the result of loss of a negative regulator. The pattern of reactivity of the monoclonal antibody TS2/7 with eight interspecies lymphocyte hybrid lines whose human parent was the leukemic line MOLT-4 suggested possible chromosomal map positions on either human chromosome 10 or 15 (Table III). This antigen could also be detected on a small subpopulation of hybrid cell line M3H8.A-1A5.SR8$^+$. However, repeated attempts to generate homogeneous TS2/7-positive cell populations from different hybrid cell lines by the FACS resulted in the loss of TS2/7 expression. This suggests that additional genetically unlinked loci may be involved in the expression of this antigen. It is also possible that hybridization stimulates only a transient TS2/7 expression on the hybrid cell populations examined, or that TS2/7 expression is diminished as a result of interactions with the mouse genome. Such epigenetic contributions would make it difficult to assign a chromosomal map position for the TS2/7 locus.

The 160,000–165,000 M_r component present in the A-1A5-defined trimeric complex (dimeric on MOLT-4) has yet to be defined by a specific antibody and therefore has not been directly examined. However, it appears that the 160,000

M_r protein on MOLT-4 originates from a genetic locus not on chromosome 10, since this subunit is no longer immunoprecipitated by A-1A5 on hybrid cells that retain this chromosome. This result is consistent with the finding that the 160,000–165,000 M_r protein is immunologically distinct from the 125,000 M_r protein, insofar as only the latter bears the A-1A5-binding epitope (10). We cannot rule out the possibility that epigenetic factors similar to those postulated for TS2/7 antigen expression may control expression of the 165,000 M_r protein and result in absence of expression despite retention of the appropriate genetic locus. These questions will be addressed when a specific antibody to this determinant is available.

At present only three antigens are known to be relatively specific for expression on activated T cells. They are the 160,000 and 210,000 dalton subunits of the A-1A5-defined complex described above, and the IL-2 receptor of 50,000–55,000 M_r (8, 19, 20). It is likely that these antigens play an important role in the activation of T cells. Thus, the genetic assignment of the A-1A5 locus to human chromosome 10 provides information that may play a role in defining events involved in the processes of cellular activation. The use of these new lymphocyte hybrids to map genes and determine the linkage relationships of complex antigenic structures extends the applications of somatic cell genetics to the cell surfaces of human T lymphocytes.

Summary

The genes that code for the human lymphocyte cell surface determinants defined by monoclonal antibodies A-1A5 and A-3A4 have been genetically mapped. All human chromosomes, except Y, were included in a series of human < mouse lymphocyte hybrid populations that retained expression of lymphocyte-specific surface markers. Expression of the A-1A5 and A-3A4 antigens was quantitated by indirect immunofluorescence and fluorescence-activated cell sorter (FACS) analysis. Hybrid populations heterogeneous for antigen expression were sorted to yield antigenically homogeneous subpopulations. Isozyme analysis indicated concordant segregation of the A-1A5 determinant with chromosome 10, and the A-3A4 determinant with chromosome 4. In contrast to the unhybridized human parent cell line (MOLT-4), from which A-1A5 immunoprecipitated two proteins (160,000 and 125,000 M_r), A-1A5 only immunoprecipitated a single band (125,000 M_r) from an A-1A5-expressing human < mouse hybrid. The genetic disassociation of these two proteins from the A-1A5-reactive complex suggests that the appearance of the 160,000 M_r protein requires a gene locus that is unlinked to the locus for the 125,000 M_r protein on chromosome 10. A third component of the A-1A5-reactive protein complex (210,000 M_r), which is recognized by the monoclonal antibody TS2/7, was not expressed on the parent MOLT-4 cells, but was weakly expressed on MOLT-4 < mouse BW5147 hybrids. This allowed preliminary mapping of that determinant to either chromosome 10 or 15. The A-3A4 antigen (~45,000 M_r) is a novel cell surface structure expressed on all hematopoeitic cell lines tested, and represents the first cell surface marker mapped to chromosome 4.

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References

1. Weiss, M. C., and H. Green. 1967. Human-mouse hybrid lines containing partial complements of human chromosomes and functioning human genes. *Proc. Natl. Acad. Sci. USA.* 58:1104.
2. Puck, T. T., P. Wuthier, C. Jones, and F.-T. Kao. 1971. Genetics of somatic mammalian cells: lethal antigens as genetic markers for the study of human linkage groups. *Proc. Natl. Acad. Sci. USA.* 68:3102.
3. Dorman, B. P., N. Shimizu, and F. H. Ruddle. 1978. Genetic analysis of the human X chromosome in human < mouse hybrids. *Proc. Natl. Acad. Sci. USA.* 75:2363.
4. Goodfellow, P., G. Banting, R. Levy, S. Povey, and A. McMichael. 1980. A human X-linked antigen defined by a monoclonal antibody. *Somatic Cell Genet.* 6:777.
5. van Someren, H., A. Westerveld, A. Hagemeijer, J. R. Mees, P. Meera-Khan, and O. B. Zaalberg. 1974. Human antigen and enzyme markers in man-Chinese hamster somatic cell hybrids: evidence for synteny between the HLA, PGM3, ME1, and IPO-B loci. *Proc. Natl. Acad. Sci. USA.* 71:962.
6. Peters, P. M., M. E. Kamarck, M. E. Hemler, J. L. Strominger, and F. H. Ruddle. 1982. Genetic and biochemical characterization of a human surface determinant on somatic cell hybrids: the 4F2 antigen. *Somatic Cell Genet.* 8:825.
7. Schroder, J., B. Nikinmoa, P. Kavathas, and L. A. Herzenberg. 1983. Fluorescence-activated cell sorting of mouse-human hybrid cells aids in locating the gene for the Leu7 (HNK-1) antigen to human chromosome 11. *Proc. Natl. Acad. Sci. USA.* 80:3421.
8. Hemler, M. E., C. F. Ware, and J. L. Strominger. 1983. Characterization of a novel differentiation antigen complex recognized by a monoclonal antibody (A-1A5): unique activation-specific molecular forms on stimulated T cells. *J. Immunol.* 131:334.
9. Cotner, T., J. M. Williams, L. Christenson, H. M. Shapiro, T. B. Strom, and J. L. Strominger. 1983. Simultaneous flow cytometric analysis of human T cell activation antigen expression and DNA content. *J. Exp. Med.* 157:461.
10. Hemler, M. E., F. Sanchez-Madrid, T. J. Flotte, A. M. Krensky, S. J. Burakoff, A. K. Bhan, T. A. Springer, and J. L. Strominger. Glycoproteins of 210,000 and 130,000 M_r on activation T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J. Immunol.* In press.
11. Peters, P. M., M. E. Kamarck, and F. H. Ruddle. 1983. Production of interspecies T cell hybrids which retain differentiation-specific surface antigens. *J. Immunol. Methods.* 63:39.
12. Hemler, M. E., and J. L. Strominger. 1982. Characterization of the antigen recognized by the monoclonal antibody (4F2): different molecular form on human T and B lymphoblastoid cell lines. *J. Immunol.* 129:623.
13. Wang, H. C., and O. S. Federoff. 1971. Banding in human chromosomes treated with trypsin. *Nat. New Biol.* 235:52.
14. Nichols, E. A., and F. H. Ruddle. 1973. A review of enzyme polymorphism, linkage, and electrophoretic conditions for mouse and somatic cell hybrids in starch gels. *J. Histochem. Cytochem.* 21:1066.
15. Harris, H., and D. A. Hopkinson. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North Holland Publishing Co., Amsterdam.
16. Meera Khan, P. 1971. Enzyme electrophoresis on cellulose acetate gel: zymogram

pattern in man-mouse and man-Chinese hamster somatic cell hybrids. *Arch. Biochem. Biophys.* 145:470.

17. Kamarck, M. E., J. A. Barbosa, and F. H. Ruddle. 1982. Somatic cell genetic analysis of HLA-A,B,C and human beta-2-microglobulin expression. *Somatic Cell Genet.* 8:385.
18. Owerbach, D., D. Doyle, and T. B. Show. 1977. Genetics of the cell surface: assignment of genes coding for external membrane proteins to human chromosomes 10 and 14. *Am. J. Hum. Genet.* 29:84*a*. (Abstr.)
19. Hemler, M. E., B. Malissen, N. Rebai, A. Liabeuf, C. Mawas, J. M. Kourilsky, and J. L. Strominger. 1983. A 55,000 dalton surface antigen on activated human T lymphocytes defined by a monoclonal antibody. *Hum. Immunol.* 8:153.
20. Leonard, W. J., J. M. Depper, T. Uchiyama, K. A. Smith, T. A. Waldman, and W. C. Greene. 1982. A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor: partial characterization of the receptor. *Nature* (*Lond.*). 300:267.