

Fluorescence-activated cell sorting of mouse-human hybrid cells aids in locating the gene for the Leu 7 (HNK-1) antigen to human chromosome 11

(gene mapping/monoclonal antibody/natural killer cells)

JIM SCHRÖDER*, BARBRO NIKINMAA*, PAULA KAVATHAS†, AND LEONARD A. HERZENBERG†

*Folkhälsan Institute of Genetics, Helsinki, Finland; and †Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

Contributed by Leonard A. Herzenberg, March 21, 1983

ABSTRACT Leu 7 (HNK-1) is a membrane antigen expressed on human natural killer cells and some other lymphoid cells. Starting with two clones of mouse-human hybrid lymphoid cells that had 1.6% and 35% Leu 7-positive cells, respectively, we viably sorted Leu 7-positive and -negative cells using a fluorescence-activated cell sorter (FACS). Short-term progeny of the sorted cells were then karyotyped. Chromosome 11 was the only human chromosome that was absent from the Leu 7-negative population and present in nearly all of the progeny of the Leu 7-positive selected cells. Thus, we assigned the Leu 7 gene to chromosome 11.

Despite the large number of human genes assigned to specific chromosomes and chromosome regions during the past decade, few coding for cell surface differentiation antigens have been mapped. This is due to several reasons: the difficulty in detecting these antigens with conventional antisera; the amount of labor needed for scoring by fluorescence microscopy; the chromosomal instability of interspecific cell hybrids; and the lack of expression of such antigens in cell hybrids made with parental cells in nonsimilar states of differentiation.

A few genes coding for human cell surface antigens that are not cell-type specific have been assigned to chromosome 11 (1, 2). Recently, Goodfellow *et al.* (3) mapped a gene coding for a cell surface antigen (F10.44.2) to human chromosome 11 using somatic cell hybrids and a monoclonal antibody. Their assignment was made by correlating, in a number of mouse-human hybrids from which some human chromosomes had been lost, the fraction of cells having particular surface antigens with the fraction of cells containing particular chromosomes. The assignment was then confirmed by using a fluorescence-activated cell sorter (FACS) by sorting hybrid cells positive and negative for the F10.44.2 monoclonal antibody and carrying two human chromosomes into a positive and a negative population. Only chromosome 11 was present in the population staining with the monoclonal antibody.

Using a similar approach, Messer Peters *et al.* (4) have assigned the gene coding for another cell surface antigen (4F2) to human chromosome 11. Hybrids heterogeneous for the expression of the 4F2 antigen were sorted with the FACS into populations homogeneous for the expression or lack of expression of the antigen. Isoenzyme analysis indicated selection for or against the presence of chromosome 11 concordant with selection for or against the presence of the antigen. The assignment was confirmed by the study of a clone containing only human chromosome 11.

We have made hybrids between human T lymphocytes and the mouse thymoma line BW5147 and used them to map the

gene coding for the T-cell sheep erythrocyte receptor to human chromosome 6 (5). In this paper we have used these same hybrids to map the gene coding for the Leu 7 or HNK-1 antigen that is found on the surface of natural killer cells and killer cells. However, instead of extensively analyzing a large number of hybrids, we selected several unstable hybrids that had both Leu 7-positive and -negative cells and many human chromosomes. With the FACS we sorted positive and negative cells. These populations were reanalyzed with the cell sorter and karyotyped both prior to and after sorting. The expression of the Leu 7 antigen was found to be correlated to the presence of human chromosome 11.

MATERIALS AND METHODS

Mouse-Human T-Cell Hybrids. Concanavalin A-activated human peripheral blood lymphocytes were fused with a hypoxanthine/aminopterin/thymidine-sensitive mouse AKR thymoma cell line (BW5147) in the presence of polyethylene glycol (5). The hybrids were isolated in selective culture medium [Dulbecco's modified Eagle's medium (GIBCO)/10% fetal calf serum (GIBCO)/0.1 mM hypoxanthine/0.4 μ M aminopterin/13 μ M thymidine] and cloned by limiting dilution on fibroblast feeders. One clone (28-4) was further subcloned. After hypoxanthine/aminopterin/thymidine selection, all mouse-human lymphocyte hybrids and the mouse BW5147 cell line were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum at 37°C in a 10% CO₂/90% air humidified atmosphere. All culture media were supplemented with penicillin (100 μ g/ml) and streptomycin (100 units/ml).

Immunofluorescence and Cell Sorting. The immunofluorescence staining was carried out as a two-step procedure by using a monoclonal antibody, anti-Leu 7 (anti-HNK-1), as the first step and an affinity chromatography-purified fluorescein-conjugated rabbit anti-mouse IgG antibody (TAGO, Burlingame, CA) as the second step reagent. The monoclonal antibody has been described by Abo *et al.* (6) and was obtained from the Becton Dickinson Monoclonal Center.

All samples were analyzed with both a FACS IV and a fluorescence microscope. One clone (28-4) and its subclone (28-4-400E8) were reanalyzed with the cell sorter 3 wk later, and both were sorted into a Leu 7-positive and a Leu 7-negative fraction. After 5 days of culture, the four fractions were reanalyzed with the cell sorter.

Chromosomal Analysis. Chromosome preparations were made by conventional methods, G-11 bands obtained by the method of Wyandt *et al.* (7), and G bands, by a modification of the trypsin-Giemsa banding method (8). The clones were screened for the presence of human chromosomes after G-11 banding, after

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: FACS, fluorescence-activated cell sorter.

Table 1. Human chromosomes present in clones 28-4 and 28-4-400E8 before and after sorting for Leu 7-positive and -negative cells with FACS IV

Clone	Number of human chromosomes present in mouse-human T-cell hybrids/20 cells studied																						%																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	fluorescent cells*																					
28-4																																														
Unseparated																							15	16	10	4		8	6		10											16	6	35		
Positive fraction†																							17	18	7			13	14		7											6	1	94		
Negative fraction†																							19	16	11	8		10											8		11	1	<1			
28-4-400E8																																														
Unseparated																							14	19	9			14											11			14			1.6	
Positive fraction†																							16	20	15	2		19	15		13											17			76	
Negative fraction†																							11	18	10	2		12											7			1	12			<1

* Expression of Leu 7 antigen was determined by indirect immunofluorescence with a FACS.

† Cells were cultured for 5 days after separation.

which 20 G-banded cells were karyotyped from each clone or subclone.

The chromosome preparations and the immunofluorescence were done on the same day because the karyotype of interspecific hybrids is not stable, with mouse-human lymphocyte hybrids slowly losing their human chromosomes. Therefore, the karyotype in a given clone can vary from cell to cell. Most mouse chromosomes in our clones and subclones can be identified as normal, although some marker chromosomes are present (5).

RESULTS

Six mouse-human hybrid clones derived from a fusion of the mouse lymphoma line BW5147 with human concanavalin A-activated peripheral blood cells were analyzed for staining with the anti-Leu 7 monoclonal antibody by both fluorescence microscopy and flow cytometry with a FACS. Three of the clones were positive with Leu 7, which had not been previously mapped. Two of these had 1.6% and 35% Leu 7-positive cells, respectively. These were then viably and aseptically sorted into positive and negative populations, which were then cultured for 5 days and reanalyzed with the FACS. Metaphase spreads were made on the days of sorting and reanalysis. The karyotype data from 20 mitoses and the fluorescence percentages shown in Table 1 show a very clear correlation of Leu 7 with chromosome 11. No other chromosome shows a positive correlation in distribution with Leu 7. Therefore, we assign the gene for Leu 7 to chromosome 11. This conclusion is also supported by the conventional concordance analysis of correlation of Leu 7 antigen expression with chromosomes present in six hybrid clones (see Table 2).

Of six clones selected for the presence of different human

chromosomes, three showed reactivity with the anti-Leu 7 antibody, whereas the remaining three and the mouse parental line were completely negative. When analyzed under a microscope, 42% of the cells of clone 28-4, 18% of the cells of clone 28-1, and 9% of the cells of clone 28-4-400E8 were positive for the Leu 7 antigen. The corresponding figures when analyzed with the FACS were 66%, 28%, and 16%. The presence of human chromosome 11 correlated best with the expression of Leu 7 antigen in these clones (Table 2), whereas several other chromosomes showed some cosegregation with this antigen. Clone 28-4 had chromosome 11 in 11 of 20 cells (55%) (Fig. 1), clone 28-1, in 4 of 20 cells (20%), and clone 28-4-400E8, in 2 of 20 cells (10%). None of the three negative clones carried human chromosome 11 in any of the 20 cells analyzed.

DISCUSSION

We have assigned the gene for a differentiation antigen (Leu 7) of human natural killer cells to chromosome 11 using a rapid method employing cell sorting and chromosome analysis. This method overcomes the serious problem of continuing chromosome loss in making gene assignments when using somatic cell hybrids. In the conventional methods, panels of hybrids with one or a few human chromosomes must be maintained by continual subcloning and karyotyping. Then phenotypes of the gene to be mapped are scored in the many clones of a panel. Because of the instability, perfect correlations are seldom obtained, requiring large numbers of cells to be analyzed. By obtaining populations virtually pure for the marker and its corresponding chromosome, assignments can be made unambiguously with a small number of cells karyotyped. A clear example of this was a clone that had only 1.6% Leu 7-positive cells prior to sorting and 76% positive upon reanalysis of the positive

Table 2. Human chromosomes present in mouse-human T-cell hybrids and proportion of cells expressing Leu 7 antigen

Clone	Number of human chromosomes present in mouse-human T-cell hybrids/20 cells studied																						%																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	Micr.	FACS																				
28-4																							8	18	17	9	8	14	11	1	12											13	5	42	66	
28-1	14	11	3	15	20	16	1	10	4	1	9											10	6	11	18	28																				
28-4-400E8																							10	17	15			14	2		8													12	9	16
28-5																							1	12	13			2	1													3	5	10	0	0
28-6																							1	1	14	16		8											5			13	8	0	0	
7-1																									12													0	0							

* Expression of Leu 7 antigen was determined by indirect immunofluorescence with a fluorescence microscope (Micr.) and a FACS.

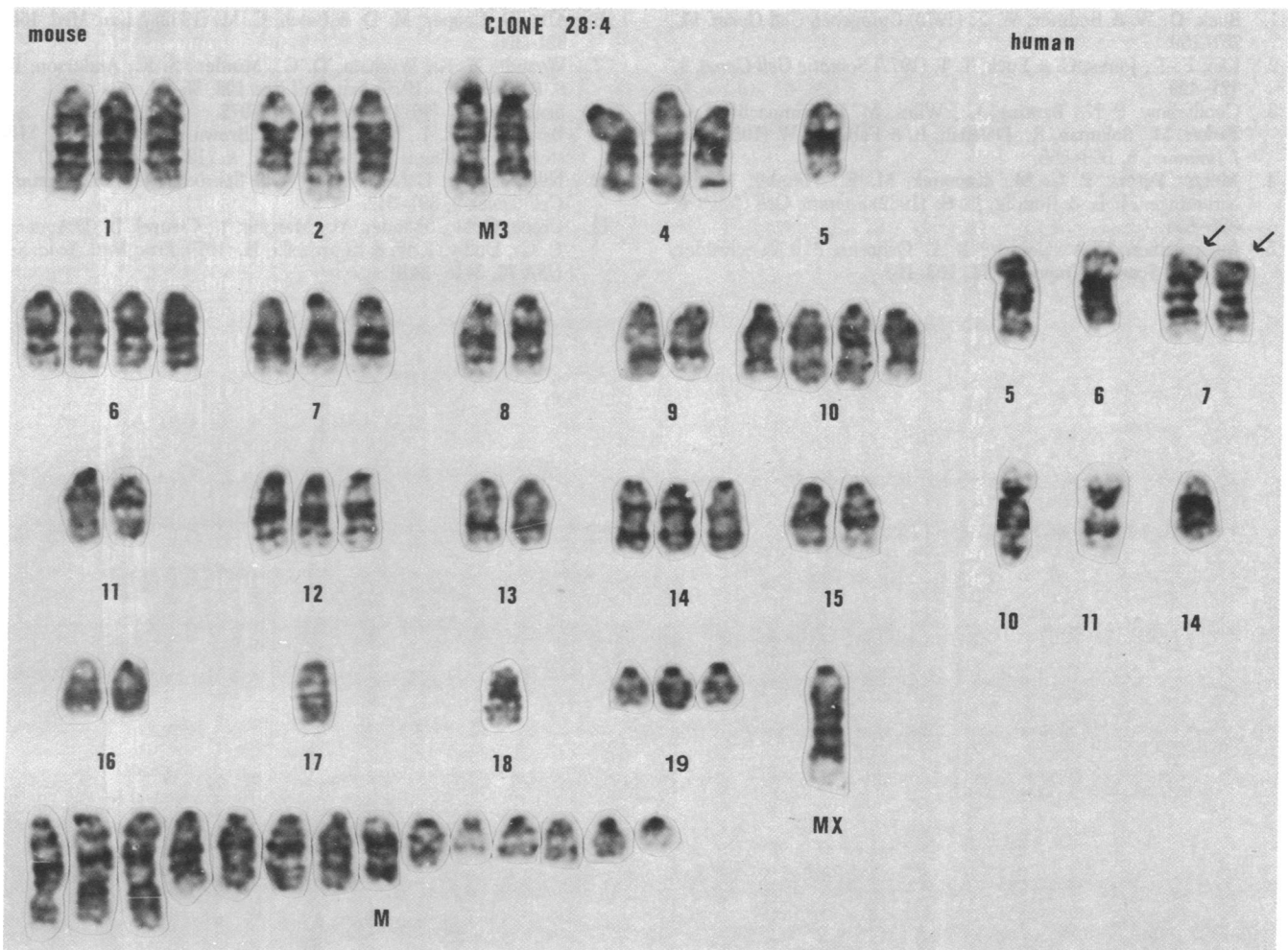


FIG. 1. A Giemsa-banded karyotype from clone 28-4. M stands for marker chromosomes, and arrows indicate deleted human chromosome 7.

fraction 5 days after sorting. At this point, 15 of 20 metaphase spreads of the latter population showed chromosome 11. Of 20 studied from the negative sorted population, no metaphases, from this clone and a second clone studied, had chromosome 11.

Interestingly, the genes involved in the biosynthesis of several other cell surface antigens have been assigned to human chromosome 11. These include glycolipids (1, 2, 9) as well as glycoproteins (3, 4, 10). The relation between the glycolipids associated with chromosome 11 is unclear, although it appears that those studied by Buck and Bodmer (1) and Kao *et al.* (2) represent different antigens. However, the mapped protein antigens coded for by genes on human chromosome 11 are evidently all different. They have all been assigned by using highly specific monoclonal antibodies, and both the tissue distributions and the molecular weights of these antigens are different. The F10.44.2 antigen described by Goodfellow *et al.* (3) has a M_r of 105,000, and it is present on most peripheral blood lymphocytes, monocytes, granulocytes, most bone marrow cells, and some nonlymphoid cells. The antigen described by Messer Peters *et al.* (4) consists of two subunits, one with a M_r of 100,000 and the other with a M_r of 41,000. This antigen is associated with cell proliferation and is present on hematopoietic stem cells and cell lines, monocytes, and activated T and B cells. The antigen described by Nikinmaa *et al.* (10) has a M_r of 75,000, and it is expressed on all peripheral leukocytes, fibroblasts, and most human cell lines. The Leu 7 antigen is present on human killer

and natural killer cells, but it is not expressed on human T or B lymphocytes, other blood cells, or on a large variety of lymphoid cell lines. It is present on HSB-2, which was the immunizing cell line for the hybridoma producing anti-Leu 7. Its M_r is $\approx 110,000$ (L. L. Lanier, personal communication).

Thus, human chromosome 11 carries genes for a group of cell surface antigens. Chromosome 6, with the major histocompatibility complex of loci, and chromosome 14, with the immunoglobulin heavy chain genes (11), are the other large clusters of genes coding for cell surface antigens. Whether the chromosome 11 genes are clustered and the significance of the chromosome 11 genes remain to be seen.

Today a large number of monoclonal antibodies are available that recognize various cell surface differentiation markers. It is now possible to use mouse-human somatic cell hybrids that express these antigens to rapidly assign genes controlling them to human chromosomes. Suitable hybrids, obtained only a few weeks after the initial fusion, without the requirement for cloning, can be analyzed for reactivity with monoclonal antibodies. Populations containing <1% to >99% of cells with a particular marker can be sorted and karyotyped, providing a rapid and accurate gene assignment to a human chromosome.

This work was supported by grants from the Finnish National Research Council for Natural Sciences, the Sigrid Jusélius Foundation, and the Ella and Georg Ehrnrooth Foundation and Grants GM-17367 and CA-04681 from the National Institutes of Health. P.K. is a National Institutes of Health Postdoctoral Fellow (5 F32 AI06343).

1. Buck, D. W. & Bodmer, W. F. (1975) *Cytogenet. Cell Genet.* **14**, 257–259.
2. Kao, F.-T., Jones, C. & Puck, T. T. (1977) *Somatic Cell Genet.* **3**, 421–429.
3. Goodfellow, P. N., Banting, G., Wiles, M. V., Tunnacliffe, A., Parkar, M., Soloman, E., Dalchau, R. & Fabre, J. W. (1982) *Eur. J. Immunol.* **8**, 659–663.
4. Messer Peters, P. G. M., Kamarck, M. E., Hemler, M. E., Strominger, J. L. & Ruddle, F. H. (1982) *Somatic Cell Genet.* **8**, 825–834.
5. Suomalainen, H. A., Goldsby, R. A., Osborne, B. A. & Schröder, J. (1980) *Scand. J. Immunol.* **11**, 163–168.
6. Abo, T., Cooper, M. D. & Balch, C. M. (1982) *J. Exp. Med.* **155**, 321–326.
7. Wyandt, H. E., Wysham, D. G., Minden, S. K., Anderson, R. S. & Hecht, F. (1976) *Exp. Cell Res.* **102**, 85–94.
8. Seabright, M. (1971) *Lancet* *ii*, 971–972.
9. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, B., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14**, 9–20.
10. Nikinmaa, B., Gahmberg, C. G. & Schröder, J. (1983) *Somatic Cell Genet.* **9**, 301–312.
11. Croce, C. M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, G. G., Dolby, T. W. & Koprowski, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3416–3419.