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Lambda-Chain Production in Human Lymphoblast–Mouse Fibroblast Hybrids

(immunoglobulins/gene localization/chromosome E-17/HAT selection/differentiation)

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ABSTRACT **Mutant human lymphoblast cells deficient in hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) activity were hybridized with thymidine kinase (EC 2.7.1.21)-deficient mouse fibroblasts. Hybrid cells were readily selected, as both parental lines were nonreverting and eliminated by hypoxanthine–amethopterin–thymidine medium. Human lambda (λ) chain was the only immunoglobulin chain produced by the lymphoblast parent, as determined by immunofluorescent techniques. Two independent hybrid clones chosen for detailed study synthesized human λ chain, and continued to do so after prolonged culture.**

As in both parental lines, no human immunoglobulin heavy chains, complement C3 or C4, or α_1-antitrypsin, or mouse immunoglobulin chains or complement C5 were detectable in the hybrids. Selection against thymidine kinase-containing hybrid cells with 5-bromodeoxyuridine did not eliminate positive λ-chain reactivity, suggesting that the kinase and λ-chain loci are not linked.

The continued production of an immunoglobulin chain by human lymphoblast–mouse fibroblast hybrids contrasts with the extinction of other differentiated functions in several hybrid systems, and indicates that gene localization and linkage analysis for human immunoglobulin chains should be feasible with this system.

Somatic hybridization with cultured mouse, hamster, and rat cells has proved a powerful tool for delineating the cellular control of differentiated functions (1) and for assigning human gene products, predominantly enzymes, to specific chromosomes or linkage groups (2). The linkage relationships and cellular control of differentiated *human* functions have been more difficult to define in view of the limited availability of human cell lines with differentiated phenotypes and the frequent extinction of differentiated functions in hybrids made from dissimilar parents (3–10).

Human lymphoblast cell lines are particularly attractive for hybridization studies, as they do not senesce in culture (11), remain largely diploid in chromosome constitution (12, 13), can be established from patients with genetic diseases as well as from normal individuals (14), carry the histocompatibility antigens of the HL-A locus as genetic markers (15), and elaborate several specialized products, including immunoglobulins, mediators of cellular immunity, interferon, and the third component of complement (C3) (16, 17). These lines all contain Epstein–Barr virus (18) or Epstein–Barr virus DNA in latent form (19–21). Recently, from one such lymphoblast line, clonal variants lacking hypoxanthine phosphoribosyltransferase (HPRT), and therefore potentially suitable for somatic hybridization with the hypoxanthine–amethopterin–thymidine (HAT) selection medium (22), have been isolated by drug selection of both untreated and mutagenized cell populations (23).

Detection of lymphoblast-specific products in hybrid cells would permit gene localization, linkage analysis, and genetic analysis of the control of these activities by available selection and chromosome techniques. Here we report the isolation and characterization of hybrid cells selected from the fusion of mutant human lymphoblast cells and mouse fibroblasts and the continued production of human lambda (λ) chain by two hybrid clones.

MATERIALS AND METHODS

Cells. The mouse fibroblast line resistant to 5-bromodeoxyuridine (BrdU) and deficient in thymidine kinase (EC 2.7.1.21) designated 3T3C2 (24), was kindly supplied by Dr. C. Basilico and used in our hybridizations. This line grows attached to tissue-culture petri dishes but not in suspension, is killed by HAT medium, and has only rare metacentric chromosomes (25). No revertants of this line have been detected in repeated selections with HAT medium, both with and without prior chemical mutagen treatment (unpublished data, S.O.).

Human lymphoblasts used in the hybridizations were derived from cells of line PGLC 33H, initiated by Dr. P. Glade from a female patient with infectious mononucleosis (26). The lines successfully hybridized in our laboratories included PGLC 33H (uncloned); L33-6-1, a clone isolated from PGLC 33H (23); and T5-1, a 6-thioguanine-resistant, HPRT-deficient variant isolated from L33-6-1 (23). As previously reported, these lines grow only in suspension or agar, and have a near-diploid chromosome constitution. Only the T5-1 line, by virtue of its HPRT-deficiency, is killed by HAT selection. No revertants of this line have been detected in 1.5×10^7 cells (23).

Mouse cells were maintained in Eagle's minimal medium supplemented with 15% fetal-calf serum. Lymphoblast lines were maintained as described (23).

Somatic-Cell Hybridization. 1×10^6 mouse cells and 6×10^6 lymphoblast cells were suspended together in 1 ml of Hank's balanced salt solution plus 0.2 ml of fetal-calf serum, incubated at 4° for 5 min, shaken at 37° for 30 min, centrifuged, and

Abbreviations: HAT, hypoxanthine-amethopterin-thymidine; λ chain, lambda light chain; HPRT, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8).

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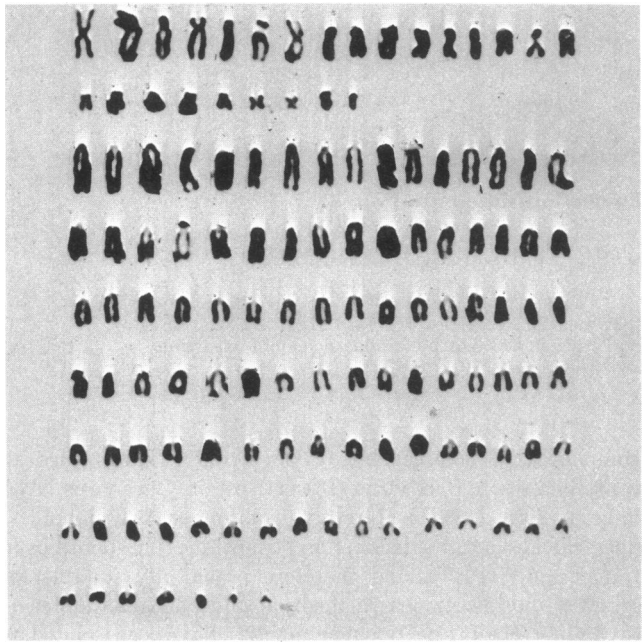

FIG. 1. Karyotype of an OV3 hybrid cell containing 128 chromosomes, 25 of which are biarmed and presumably almost all of human origin. In addition, some of the telocentric chromosomes may be human.

suspended in fresh medium containing 15% fetal-calf serum. Aliquots of this suspension were plated for selection of hybrids with HAT medium. In some experiments 1000 HAU (hemagglutinating units) of β-propiolactone-inactivated Sendai virus was present.

24 hr after the cell mixture was plated, lymphoblast cells were seen adherent to mouse cells attached to the culture surface. When PGLC 33H or L33-6-1 was used as the lymphoblast partner, the monolayers were rinsed repeatedly with phosphate-buffered saline (pH 7.4) for the first 3 days of selection with HAT medium to remove floating lymphoblast cells as completely as possible. When the HPRT-deficient T5-1 line was used, such washing was not necessary since HAT selection completely eliminated T5-1 cells.

Within 10 days after plating and HAT selection, macroscopic colonies were visible on the culture dishes at a frequency of 3×10^{-4} (based on the number of mouse cells originally plated), irrespective of the presence or absence of a Sendai virus preparation active in promoting fusion of hamster cells. HAT selection at the time of cell plating or 24 or 48 hr later resulted in the same yield of hybrid colonies. Clones could be isolated with stainless steel cylinders after 14 days, and were maintained in Eagle's medium after an initial passage in HAT medium.

Preparation of Fluorescent Antisera. Procedures for isolation of myeloma proteins, Bence Jones proteins, and IgM macroglobulins, and for preparation of heavy and light chains and papain and pepsin digestion fragments, have been reported (27, 28). Antisera polyvalent for human immunoglobulins and antisera specific for human immunoglobulin classes, IgG subclasses, and light chain types were prepared by immunization of rabbits, goats, cynomolgus and rhesus monkeys, and baboons, and their specificity was ascertained (29, 30). Antisera to human complement components C3 and C4 were obtained from Hyland Laboratories. Antiserum to mouse C5, prepared in a strain deficient in C5 (31, 32), was the kind gift of Dr. L. T. Rosenberg. Polyvalent and class-specific antisera to mouse immunoglobulins were obtained from Meloy Laboratories and from Drs. J. Schwab and J. Folds. Antisera were fluoresceinated or rhodoaminated, and, when necessary, conjugates were chromatographed on DEAE-cellulose to obtain fractions with optimum fluorescein:protein ratios (30).

Specificity of each antiserum was established by the following criteria: (*i*) Ouchterlony double diffusion, (*ii*) hemagglutination by the fluorescent conjugate of human type O, Rh-positive erythrocytes to which purified myeloma proteins were coupled with bis-diazotized benzidine (33), (*iii*) blockage of fluorescence by prior incubation with unconjugated antiserum, and (*iv*) blockage of fluorescence only by purified proteins of the designated specificity.

Immunofluorescent Staining Techniques. For cytoplasmic staining, coverslips from cultures of 3T3C2 cells and hybrids, and suspensions of lymphoblasts, were washed four times in phosphate-buffered saline and twice in phosphate-buffered saline with 5% Mayer's albumin fixative. 50 μl of lymphoblast cell suspension (5×10^6 cells per mm^3) was applied to a slide. Slides were air-dried and fixed in alcohol–acetone 1:1 for 10 min. All slides and coverslips were incubated for 30 min with specific antisera and washed three times in phosphate-buffered saline for 10 min.

Surface immunoglobulins on the lymphoblasts were stained essentially as described by Pernis *et al.* (34). Cells were washed in Hank's medium four times and Hank's medium with 5% Mayer's albumin fixative, and the cell suspension was incubated with an equal volume of specific antiserum for 30 min. Antisera and controls were centrifuged before use to remove aggregated material. The cells were then washed three times in Hank's medium plus bovine-serum albumin, suspended in 50 μl of the same suspension, put on slides, and observed or allowed to dry and mounted with phosphate-buffered glycerol (pH 7.2). Although lymphoblasts could be stained with either cytoplasmic or surface techniques, clearer distinction between positive and negative cells and brighter fluorescence was obtained with surface staining, so that most determinations on lymphoblast lines were done by this method.

The preparations were examined in a Leitz Orthoplan fluorescent microscope with an Osram HBO-200 mercury vapor lamp, Plöem vertical illuminator with the appropriate combinations of excitor filters, dichroic mirrors, and barrier filters for either fluorescein or rhodamine. For each specimen, about 1000 cells were examined for staining. For each experiment, antiserum absorbed with purified immunoglobulins of the designated specificity was used as a control. All cell lines were tested with polyvalent antiserum, as well as with all specific antisera available.

RESULTS

Human–Mouse Nature of Hybrids. Two independently derived human lymphoblast–mouse fibroblast hybrid clones (V1 and OV3) originating from fusion of T5-1 and 3T3C2 cells were chosen for studies on lymphoblast-specific products. Hybrids were also isolated with PGLC 33H and L33-6-1 lymphoblast cells as human parents. Hybrid V1 was obtained from a hybridization experiment in which Sendai virus was present initially, and OV3 from one without virus. Glaser and

O'Neill (35) have reported hybridization of Burkitt lymphoblast cells and mouse fibroblasts, using selection against only the fibroblastic parent.

Chromosome analyses established the human–mouse nature of the putative hybrids. T5-1 has a predominantly diploid, 46 XX karyotype (23), while 3T3C2 is a hypotetraploid line with 65–68 telocentric chromosomes and rare biarmed chromosomes (ref. 25; and unpublished data, S.O. and P.B.). Hence, biarmed chromosomes observed in presumptive hybrids represent almost exclusively human chromosomes. Chromosome counts on parental lines and on V1 and OV3 hybrids are shown in Table 1, and a representative metaphase in Fig. 1. Similar high chromosome numbers have been reported in other 3T3–human hybrids (25), and may reflect either duplication of the mouse chromosome complement or fusion of more than two cells (36).

Hybrids were uniformly larger and more elongated than 3T3C2, more closely resembling human fibroblasts. All grew slowly in either HAT or regular medium immediately upon isolation; but within 2 months they grew almost as rapidly as the mouse parent, as reported for human fibroblast–mouse fibroblast hybrids and correlated with elimination of human chromosomes (37).

Independent evidence for the human contribution to our hybrids was the presence of human and interspecific heteropolymeric forms of glucose-6-phosphate dehydrogenase B as well as the mouse form on acrylamide-gel electrophoresis of one PGLC 33H x 3T3C2 hyrbid clone selected and studied before the availability of the T5-1 line (unpublished data, S.O. and Dr. V. M. Riccardi).

Production of Human λ Chain by Hybrids. When first studied by Glade and Chessin soon after initiation in culture, PGLC 33H produced the heavy chains of IgG, IgM, and IgA, and the κ light chain. λ Light-chain production also was detected after 4 weeks of additional culture. Variations in chain productions for lymphoblast lines have been repeatedly observed in the early stages of culture (17, 26).

In our present stock of PGLC 33H, and in the T5-1 clone, human λ chain is the only immunoglobulin chain detectable by immunofluorescence. On repeated testing, the presence of λ chain in PGLC 33H and T5-1 has proved to be a stable property. For example, staining for surface immunoglobulins in these lines has, on average, shown PGLC 33H to have 38% positive cells (three experiments during 6 months) and T5-1 to have 45% positive cells (six determinations during 9 months). The mouse parent, 3T3C2, was uniformly negative with all antisera.

TABLE 1. *Chromosome complements of parental and hybrid cell lines*

Cell lines	Mean no. (range)*	Biarmed chromosomes
Parental		
3T3C2 (mouse)	66 (65–68)	0–2
T5-1 (human)	46 (45, 46)†	35, 36†
Hybrids		
V1	103 (92–110)	13 (7–15)‡
OV3	134 (120–156)	17 (10–26)‡

* 10–25 Cells were counted to determine each mean chromosome number.

† Of 22 cells examined, 19 were 46 XX (36 biarmed) and three were 45 XX, C-(35 biarmed).

‡ Both hybrids were studied after 14 weeks of growth in culture. The biarmed chromosomes with their ranges in parentheses represent an estimate of the minimum number of human chromosomes present.

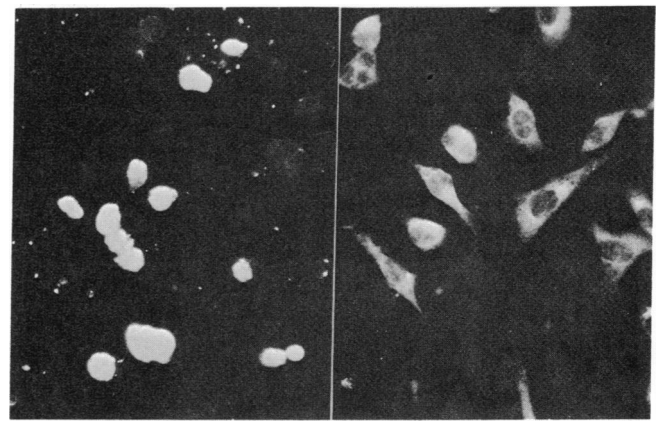

FIG. 2. Immunofluorescence of T5-1 lymphoblast (*left*) and V1 hybrid (*right*) cells with antiserum to human λ chain. Magnification ×235.

Hybrids OV3 and V1 have continued to produce human λ chain, even after 9 months of total culture time each. As in T5-1, human heavy chains of IgG, IgM, IgA, IgD, and IgE, and κ light chain were not detected at any time. OV3 had 22% cells staining with λ antisera (average of six determinations from 3–9 months of culture time, range: 7–30%) and V1, 27% positive cells (three determinations from 3–9 months of culture time, range: 10–32%). From immunofluorescence data alone no quantitative estimate of λ-chain production in the hybrids relative to that in T5-1 can be made.

Positive fluorescence patterns for T5-1 and the V1 hybrid are shown in Fig. 2. In the hybrids, rounded cells tended to give more strongly positive reactions than elongated cells. These may be cells near mitosis in which compact cellular morphology concentrates visible fluorescence. Variation in immunoglobulin-chain production during the cell cycle (38, 39) may contribute to this appearance as well.

One hybrid clone of PGLC 33H x 3T3C2 has not been observed to produce λ chain detectable by immunofluorescence (six determinations from 3–6 months culture time). Although this clone contained fewer than six biarmed chromosomes at 3 months of culture time, extensive comparison of the karyotype of this clone with either OV3 or V1 has not been undertaken, as it may have arisen from fusion of a mouse fibroblast with a nonproducing lymphoblast cell in the uncloned PGLC 33H line, and would therefore be uninformative for further investigations of linkage.

Search for Other Products in Lymphoblast–Fibroblast Hybrids. Other investigators have reported the association of mediators of cellular immunity, interferon and complement factor C3 with lymphoblast cell lines (16, 17). T5-1 and OV3 lines reacted negatively with fluorescently-labeled antisera to human complement C3 and C4. Furthermore, biologically active human complement C2, C3, and C4 were not detectable in the culture medium of growing T5-1 cells with assay systems capable of detecting less than 1 ng/ml (kindly tested by Dr. H. Colten).

Although no IgG heavy chain was detected in T5-1 or in V1 and OV3 hybrids, these lines were tested for the presence of human α_1-antitrypsin, known to be linked to the Gm locus (40). Culture medium from these lines contained less than 0.7 μg/ml of α_1-antitrypsin (after growth of cells for 1 week to confluency or stationary phase), as determined by electroimmunodiffusion (assays kindly performed by Dr. R. Talamo and Miss C. Langley and by Dr. A. M. Johnson).

In view of recent results in other systems indicating that inductions of cellular activities may occur in interspecific hybrid cells (41–43), the possibility was considered that *mouse* immunoglobulins and complement factors might have appeared in our hybrids. However, no mouse immunoglobulins, heavy or light chains, or mouse complement component C5 were detected with antisera to these products.

Absence of λ-Chain Linkage to the Human Thymidine Kinase Locus. Survival of hybrids in HAT medium is dependent on the presence of human thymidine kinase, previously assigned to human chromosome E-17 (44). Cells with thymidine kinase activity cannot survive in medium containing BrdU, and any hybrid cells capable of growth in BrdU would most likely have lost either both chromosomes 17 entirely (by segregation) or at least those parts containing the kinase loci. Therefore, OV3 and V1 hybrid cells were exposed to BrdU (0.1 mM) for 2 weeks, and the surviving cells were retested for the continued presence of λ chain. Positive reactivity to λ-chain antisera was retained, suggesting that selection against the thymidine kinase locus did not result in loss of λ-chain production.

DISCUSSION

Human lymphoblast–mouse fibroblast hybrids provide a system for analysis of the chromosomal representation and control of synthesis of immunoglobulins and related proteins. If, as seems likely, a single human chromosome is sufficient for the continued production of λ chain observed in our hybrid clones, then λ-chain production may be lost on further culture of the OV3 and V1 clones, or in subclones thereof. Assignment of the λ-chain locus to a certain human chromosome should be possible through identification of the specific chromosomes present before and after this event. This task will be more difficult if both regulatory and structural loci, perhaps nonsyntenic in the chromosome complement, are involved. With the rapid accumulation of markers assigned to various chromosomes (2), it may be easier technically to make such an assignment by demonstrating correlations between the presence of λ chain and other human proteins.

The wide range of immunoglobulin chains made by lymphoblasts (16, 17), at times more than one heavy chain per cell (45), and the ability to clone and mutagenize these lines (23), should permit the isolation of lymphoblast clones producing various immunoglobulin chains. The systematic hybridization of these cells to mouse fibroblasts should permit assignment of all immunoglobulin chains to specific linkage groups and chromosomes.

Previous work with hybrids of immunoglobulin-producing mouse-myeloma cell lines and nonproducing mouse lines has shown that immunoglobulin chains or intact immunoglobulins may (46, 47) or may not (9, 48) be produced after fusion. In one series of hybrids, production of immunoglobulins was related to the chromosome number of the hybrids (47). The continued production of λ chain in our hybrids after prolonged growth in nonselective medium probably reflects the fortuitous retention of the human chromosome (and locus) responsible, or less likely, translocation of the human locus to a mouse chromosome or selection of hybrids with multiple copies of the locus or relevant chromosome(s). We excluded linkage of λ-chain production to the thymidine kinase locus, and therefore to chromosome E-17, by selection of hybrids with BrdU, after which the surviving cells retained the ability to produce λ chain.

Since the exact rates of λ-chain production in T5-1 and our two hybrids are not known, we cannot exclude the possibility that this differentiated function is decreased in the hybrids. Still, the continued production of human chain in these clones appears to provide an example of the maintenance of a differentiated function after fusion of differentiated and nondifferentiated cells, in contrast to the more commonly reported phenomenon of extinction (1, 3–10). Other instances include synthesis of collagen and hyaluronic acid (49) and of albumin (41), production of retinal pigment in fusions of 2s melanoma cells and fibroblasts (50), expression of electrical excitability of neuroblastoma cells (51), and induction of aryl hydrocarbon hydroxylase (52). In these studies the differentiated parental lines have been represented at times by more than one chromosome complement in the hybrids, so that attention has been focused on gene dosage or balance (50). Alternatively, these instances and that of immunoglobulin production suggest the existence of classes of differentiated functions less subject to regulatory control than most inducible or tissue-specific enzymes, or expressed in a dominant fashion (52).

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