COMPLEMENT-MEDIATED ANTISERUM CYTOTOXIC REACTIONS TO HUMAN CHROMOSOME 7 CODED ANTIGEN(S): IMMUNOSELECTION OF REARRANGED HUMAN CHROMOSOME 7 IN HUMAN-MOUSE SOMATIC CELL HYBRIDS*

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Cytotoxic antibodies or lymphocytes sensitized to specific cell surface antigens have been used to select somatic cell hybrids with specific chromosome exclusions. Spontaneous loss of chromosomes derived from one of the parental cells is a characteristic of somatic cell hybrids; thus in a population of hybrids, cells can pre-exist which have lost the chromosome coding for the cell surface antigen under selection and these cells escape the specific immunologic cytotoxic reaction and grow. This phenomenon was first demonstrated with human-mouse somatic cell hybrids exposed to rabbit anti-human cell sera and complement (C) (1). Those hybrid cells which survived the cytotoxic lysis were found to be missing a human chromosome present in the original population. The chromosome coding for this antigen(s) was assigned to human chromosome 11 both by linkage studies (1, 2) and by chromosome banding techniques. Antiserum to an antigen coded for by human chromosome 11 has also been raised in mice by immunization with syngeneic human-mouse somatic cell hybrids containing human chromosome 11 (3). Rabbit anti-human β_2-microglobulin serum and complement (C) have also been utilized to selectively eliminate, from populations of human-mouse hybrid cells, those hybrids containing human chromosome 15, the chromosome that codes for human β_2-microglobulin.[1] Allo-immune lymph node and spleen cells have been used to select interstrain murine somatic cell hybrids. This cell-mediated immunoselection resulted in the growth of hybrid cells lacking the major histocompatibility complex (H-2) antigens of the parental strain, against which the cytotoxic cells were sensitized, and concurrently the number of mouse chromosome 17, known to code for these antigens, was reduced (4).

[1] Jones, E. A., P. Goodfellow, R. Kennett, and W. F. Bodmer. 1977. The independent expression of HLA and β_2-microglobulin on human-mouse hybrids. Somatic Cell Genetics. In press.

Some chromosomes are mandatorily retained in somatic cell hybrids because the products of genes contained on these chromosomes are essential for the growth of the hybrid cells. The chromosome bearing the normal gene is retained in hybrid cells when nutritional auxotrophic mutants (5) or drug-resistant mutant cells (e.g., thymidine kinase deficient or hypoxanthine-guanine-phosphoribosyl-transferase deficient) (6) are hybridized with normal cells and grown in selective medium. Obligate retention of the human chromosome 7 has also been described (7) when normal mouse peritoneal macrophages are hybridized with a simian virus 40 (SV40)[2]-transformed human cell line. The nondividing macrophage is transformed by the presence of the SV40 genes integrated on human chromosome 7 and such phenotypes of SV40-transformed cells as the expression of SV40 tumor (T) antigen (8), growth in agar (7), and tumorigenicity in nude mice (9), are expressed in these hybrid cells. No loss of human chromosome 7 has been found in these hybrid cells, for presumably cells that lose this chromosome and the integrated virus are unable to proliferate.

We have described (10) the preparation and reactivity of antisera prepared in mice to hybrids between syngeneic normal mouse peritoneal macrophages and SV40-transformed human cells. The immunizing hybrids contained, in addition to the complete murine genome, the human chromosome 7 from an SV40-transformed human skin fibroblast (LN-SV) (7) as the only human chromosome. These antisera reacted in radioimmunoassays with human fibroblastic cell lines of many origins and with those human-mouse hybrid cells that contain human chromosome 7. No evidence could be found for any reactivity to SV40-controlled tumor-associated specific antigens (SV40 TASA). This report describes the reactivity of these antisera in C-dependent lysis (CDL) and their use to immunoselect clones of hybrid cells containing rearranged human chromosomes 7. Characterization of these clones has permitted the tentative assignment of several genes to different regions of human chromosome 7.

Materials and Methods

Antisera. Antiserum to hybrid cell line 53-87-1(1) clone 21 (cl 21), a C57BL × LN-SV hybrid containing chromosome 7 as the only human chromosome, was prepared by weekly intraperitoneal injections of 2 × 10⁷ cells into C57BL/6J male mice. Two separate pools of antisera were prepared, one from a group of mice bled 10 days after eight immunizations and one from another group of mice bled 10 days after the seventh immunization. Antiserum to SV40 T antigen was obtained from tumor-bearing hamsters injected with SV40 as newborns and used at 1:30 dilution. Fluorescein isothiocyanate-labeled rabbit immunoglobulin (IgG) against hamster IgG was purchased from Cappel Laboratories, Inc., Downingtown, Pa. The rabbit serum used as a C source was obtained by repeated cardiac puncture. This C was selected after testing for spontaneous toxicity to human, mouse, and human-mouse hybrid cells. A pool of C57BL/6 antiserum to a human-mouse hybrid cell line of different chromosomal constitution was used as control. For absorption 1 ml of antiserum diluted 1:20 in Eagle's minimal essential medium (MEM) (Auto Pow; Flow Laboratories, Inc., Rockvill, Md.) supplemented with 10% fetal bovine serum (FBS) (Reheis Chemical Co., Chicago, Ill.) was mixed with a 0.5 ml packed volume of cells (2 × 10⁸ cells). The mixture was

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[2] *Abbreviations used in this paper:* CDL, complement-dependent lysis; cl, clone; FBS, fetal bovine serum; IS, immunoselected; LN-SV, SV40-transformed human skin fibroblast; PBS, phosphate-buffered saline; RCF, relative centrifugal force; RI, reactivity index; RIA, radioimmunoassay; SV40, simian virus 40; SV40 TASA, SV40-controlled tumor-associated specific antigens; T, tumor.

incubated for 1 h at 25°C with continuous shaking and for 15 h at 4°C stationary. Antisera were separated by $^{1}/_{2}$ h centrifugation at 27,000 relative centrifugal force (RCF).

Cells and Cell Cultures. The cell lines and their origins are described in Tables I and II; the two hybrid clones 53-87-1(1) cl 21 and 53-87-3 cl 36 were kindly donated by Dr. C. M. Croce, Wistar Institute. All cell lines were grown in MEM and 10% FBS. Human peripheral blood lymphocytes were separated from freshly drawn heparinized blood by density gradient centrifugation on Ficoll-Hypaque (sp gr 1.077). Cells for cytotoxic assays or indirect radioimmunoassays were plated at confluent density 18 h before testing. Single cell suspensions were prepared by washing cell monolayers three times with calcium- and magnesium-free Dulbecco's modified phosphate-buffered saline (PBS) and then exposing them for 1 min to 0.25% trypsin and 0.1% EDTA in PBS. Cells were harvested in MEM containing 10% FBS and pipetted vigorously.

Cloning was done by diluting cell suspensions in MEM and dispensing approximately 1 cell per well into a flat-bottom microtiter plate (no. 3040; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). After cell attachment, wells containing one cell were scored and only cells from these wells were harvested when confluent.

Cytotoxic Assay and Immunoselection Technique. Appropriate dilutions of antisera (0.05 ml) were added to 0.05-ml aliquots of cell suspensions (4 × 10^{6} viable cells/ml) in wells of Linbro U-bottom microtiter test plates (IS-RB-96; Linbro Chemical Co., New Haven, Conn.). The sealed plates were shaken at 37°C for $^{1}/_{2}$ h and then centrifuged at 225 RCF for 3 min, the antisera aspirated, and 0.10 ml of freshly diluted (1:10), ice-cold rabbit C added. MEM plus 10% FBS plus 10 mM Hepes was used as diluent throughout. After another 30 min of shaking at 37°C, the plate was immersed in ice and 0.1 ml of ice-cold erythrosin-B (11) added. Viability counts of between 100–300 cells were made in a hemocytometer. In addition to the antisera dilutions and C, each cell line was incubated with C alone, with normal mouse serum and C, and with the lowest dilution of antiserum alone to assess these components for nonspecific cytotoxicity. The dilution of antisera necessary to give 50% CDL (CDL titer) was calculated from the graphed results.

For immunoselections, the antisera were diluted 1:100 and the above procedure was followed except that the contents of the wells were removed aseptically after C incubation and a sample taken for counts of viable cells. The cells were then either plated in T-25 Falcon flasks or cloned directly.

Radioimmunoassay. The indirect ^{125}I-radioimmunoassay (RIA) was used as previously described (10). Triplicate determinations were made for each serum or antiserum dilution and a reactivity index (RI) was calculated for each cell line used. This index was determined by subtracting the average number of counts per minute bound to cells in dilutions of normal serum from counts per minute bound to cells incubated with an equivalent dilution of cl 21 serum and then comparing the number of counts with those bound to the human cell line LN-SV. Calculation of the RI allowed for comparison between experiments:

$$RI = \frac{(\text{cpm for cell x + antiserum}) - (\text{cpm for cell x + normal serum})}{(\text{cpm for LN-SV + antiserum}) - (\text{cpm for LN-SV + normal serum})} \times 100.$$

Chromosomes. Chromosome banding of colcemid-arrested metaphase cells was performed by the trypsin-giemsa technique (12).

β-Glucuronidase. β-glucuronidase was assayed after starch gel electrophoresis of extracts of LN-SV cells, of a BALB/c mouse embryo fibroblast, of cl 36 cells, and of cells from immunoselected subclones of cl 36 using a modification of the technique described by Chern and Croce (13).

Tumorigenicity in "Nude" Mice. Congenitally athymic nu/nu (nude) mice were produced in our colony by crossing (nu/nu) BALB/c males to C57BL/6J females and choosing nude offspring from F_{1} intercrosses. 5- to 6-wk-old nude mice were injected subcutaneously with 10^{7} cells suspended in 0.1 ml MEM plus 10% FBS. The mice were checked periodically for the appearance of tumors. 7 wk postinjection, the mice were exsanguinated and the tumors were removed and fixed in formalin for histologic examination. Each cell line was injected into four nude mice.

Results

Reactivity of Anti-cl 21 on Different Cell Lines. The CDL of anti-cl 21 serum was assessed on a variety of cell lines (Table I). The CDL titer on LN-SV and hybrid cls 21 and 36, derived from LN-SV, was 1:1,300 or greater, though a

TABLE I

Origin of Cell Lines, CDL Titer, Reactivity Index, and Expression of SV40 T Antigen

Cells	Origin of cells	CDL titer*	Reactivity index‡ (RIA)	SV40 T antigen
Human				
LN-SV	SV40-transformed skin fibroblasts	>1:1,600	100	+
HT1080	Fibrosarcoma derived	1:500	102	−
RDMc	Rhabdomyosarcoma derived	1:400	69	−
1054-TR	SV40-transformed brain cells	<1:20	61	+
W18Va2	SV40-transformed buccal mucosa cells	<1:20	71	+
HeLa (D-98)	Cervical carcinoma derived	<1:20	45	−
WI38	Embryonic lung fibroblasts	<1:200	41	−
LN	Human skin fibroblasts (not parent of LN-SV)	<1:20	40	−
Lymphocytes	Fresh peripheral blood lymphocytes	<1:200	2	−
Monkey				
GMK EVa cl 2A-1	SV40-transformed African green monkey kidney cells	<1:200	67	+
CV-1	African green monkey kidney cells	<1:200	46	−
Mouse-human hybrid				
53-87-1(1) cl 21	LN-SV × C57BL/6 mouse macrophage§	1:1,300	69	+
53-87-3 cl 36	LN-SV × BALB/c mouse macrophage§,‖	>1:1,600	69	+
53-87-3 cl 36 IS mass	Same – IS‖	1:400	65	+
53-87-3 subclone 2, 3, 6, 8, 11	Cloned from cl 36 IS mass‖	1:400	49	+
53-87-3 subclone 1, 7, 9, 14	Clones from cl 36 IS mass‖	<1:200	20	+
53-87-3 cl 36 IS subclone 2 IS mass and subclones	Derived from cl 36 IS subclone 2 IS‖	<1:200	ND	+
Rodent				
MKS-Bu-100	SV40-transformed BALB/c mouse kidney cells	<1:200	7	+
C57-SV	SV40-transformed C57BL/6 mouse embryo fibroblasts	<1:200	9	+
MC57G	Methylcholanthrene-induced tumor in C57BL/6 mouse	<1:20	9	−
F5-1	SV40-induced hamster tumor	<1:200	7	+
WIRL-SV	SV40-transformed rat liver cells	<1:200	2	+

* Antiserum dilution necessary to give 50% CDL.
† For calculation see Materials and Methods.
§ Contains intact human chromosome 7.
‖ See Table II for human chromosome 7 rearrangement.

prozone was seen at dilutions lower than 1:40 (Fig. 1). Two other human cell lines, HT1080, derived from a fibrosarcoma, and RDMc, derived from a rhabdomyosarcoma, were each susceptible to specific cytotoxic lysis by anti-cl 21 serum. Absorption of anti-cl 21 serum with HT1080 cells removed its C-dependent cytotoxic activity to LN-SV. The residual cytotoxicity is accounted for by the background cytotoxicity of C (Fig. 1). The remaining primate cells tested, both transformed (1054-TR, W18Va2, HeLa, and GMK EVa cl 2A-1) and nontransformed (LN, WI38, human peripheral blood lymphocytes, and CV-1) were not lysed with this antiserum throughout the range of dilutions that gave maximal lysis on the LN-SV cell line. No CDL of anti-cl 21 serum (above the C toxicity background) was found on any rodent cell line tested (Fig. 1 and Table I). These cells include SV40-transformed mouse (MKS-Bu100, C57SV) and rat (WIRL-SV) cells and cells derived from an SV40-induced hamster tumor (F5-1).

Comparison of the reactivity of anti-cl 21 serum in indirect ^{125}I-RIAs with its reactivity in CDL revealed some human cell lines that bound antiserum but were insusceptible to CDL. For example, RDMc, W18Va2, and 1054-TR each bind equivalent amounts of serum yet only RDMc reacts with anti-cl 21 sera to
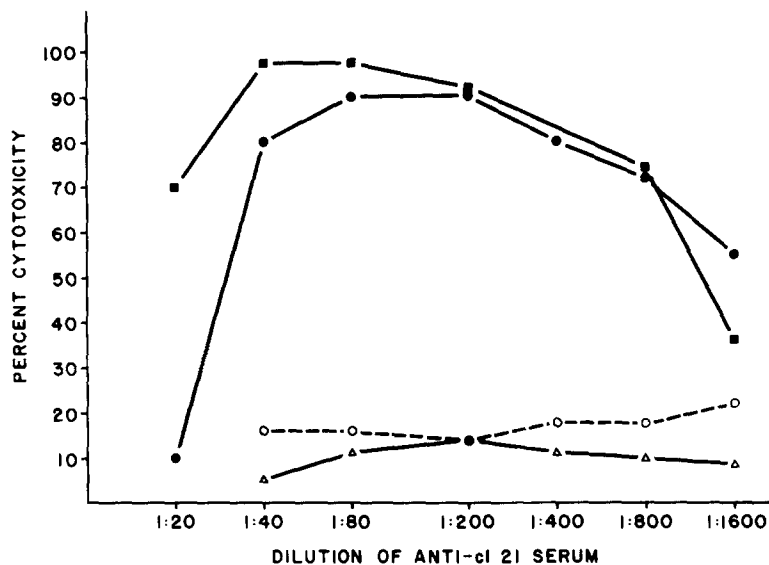
FIG. 1.   Anti-cl 21 serum CDL tested on: (■---■), cl 21 mouse-human hybrid cells; (△---△), MC57G mouse cells; (●---●), LN-SV human cells; and (○---○), LN-SV human cells after absorption with human cells.

give CDL (Table I). The susceptibility of each of the human cells lines to general CDL was assayed using rabbit C and dilutions of another mouse anti-human-mouse hybrid cell sera of different reactivity; cells of all lines, except HeLa, were lysed.

Immunoselection of cl 36.   53-87-3 cl 36, the BALB/c mouse peritoneal macrophage × LN-SV hybrid, containing human chromosome 7 in one or two copies per metaphase plate as the only human chromosome (see Fig. 2) was used for immunoselection. This hybrid cell line was maximally lysed with anti-cl 21 serum and C [CDL titer >1:1,600 (Table I)]. On immunoselection, 99% of the cells were lysed. After transfer of the immunoselected population to a T-25 culture flask, the surviving cells were allowed to grow to confluence. This immunoselected (IS) population is designated cl 36 IS mass. When the cells were tested with anti-cl 21 serum in CDL, a decrease in reactivity was seen (CDL titer, 1:400; see Fig. 3).

A series of subclones were derived from cl 36 IS mass (cl 36 IS subclones 1–14). Cl 36 IS subclones 3, 6, 8, and 11, showed the same reduced reactivity as did the cl 36 IS mass population with anti-cl 21 serum in CDL (Fig. 3; CDL titer, 1:400), while subclones 1, 7, 9, and 14, appeared essentially nonreactive (Fig. 3; CDL titer, <1:200). cl 36 IS subclone 2, containing some cells reactive in CDL (23% lysis with antiserum 1:200), was again IS as before and four mass populations and a series of secondary subclones (cl 36 IS subclone 2 IS subclone 1–8) were derived from this immunoselection. These subclones were nonreactive in CDL with the antiserum (Fig. 3).

The results from indirect RIAs (Table I) roughly parallel those obtained with cytotoxicity. The original hybrid cl 36 specifically bound 69% of the counts bound by the human parental cell line LN-SV; cl 36 IS mass gave similar

results (RI, 65%). The average RI of the cl 36 IS subclones 2, 3, 6, 8, and 11, each of which contained some cells reacting in CDL with anti-cl 21 serum, dropped to 50%. The cl 36 IS subclones that showed no reactivity in C-mediated antiserum cytotoxicity still gave an RI of 20% compared with a 7% RI to a mouse cell line tested in parallel (Table I).

Chromosome Studies. Although most of the original cl 36 hybrid cell lines contained an apparently normal human chromosome 7 (Fig. 4 A; Table II) 15% of these cells also contained a chromosome that, from the trypsin-giemsa-banding pattern, appeared to represent a Robertsonian translocation with both centromeres and the long (q) arms of the human chromosome 7 being retained (7qter → 7cen::7cen → 7qter, hereafter referred to as isoq7; see Fig. 4 C), One karyotyped metaphase showed, in addition to the normal 7 and the isoq7, a different centric fusion involving mouse chromosome 12 translocated to the pl region of the short (p) arm of the human chromosome 7 rea (HSA plus MMU) (7qter → 7 cen → 7pl::12cen → 12qter) (Fig. 4 D). 35% of the metaphases of cl 36 IS mass contained a normal human chromosome 7. 47% of the metaphases also contained a chromosome with banding patterns (Fig. 4 B) corresponding to human chromosome 7, but the ratio of the short arm:total chromosome length of this chromosome fell below 0.31, the lowest ratio found when measuring these parameters on the human chromosome 7 in the original cl 36 7del (7qter → 7pl) (Fig. 4 B). The percentage of cells containing the isoq7 remained the same as in the original clone.

A large number of metaphases from most of the subclones derived from cl 36 IS were analyzed (Table II). Subclones 1 and 7 contained the human 7-mouse 12 translocation (Fig. 4 D) and no normal human chromosome 7. This same translocation is found in 77% of the metaphases of subclone 14 and in addition, 23% of the cells contain a single human 7 with the p terminal deletion (Fig. 4 B). Subclones 2 and 9 each contained a significant number of metaphases with only the isoq7 chromosome (Figs. 2 B and 4 C), although some metaphases of subclone 9 (10%) also had a normal human chromosome 7 and some metaphases (36%) of subclone 2 contained the 7qter → 7pl chromosome.

Analysis of over 200 metaphases of derivatives of cl 36 IS cl 2 IS (three mass populations and eight subclones) revealed no normal human chromosomes 7. The subclones contained either the human 7-mouse 12 translocations and/or the human isoq7; the majority of the mass populations contained only the isoq7.

SV40 T Antigen and Tumorigenicity. Each of the clones and subclones were tested by indirect immunofluorescence with hamster antiserum specific for the SV40 nuclear T antigen and each expressed this antigen (Table I). Each of the cl 36 IS subclones and subclone 2 IS, subclones 3, 4, and 7 were injected into nude mice and all of these cell lines produced tumors (approximately 0.5 × 1.0 cm) by 7 wk after injection which were classified histologically as undifferentiated sarcomas.

β-Glucuronidase. Visualization of β-glucuronidase activity after starch gel electrophoresis indicated that the cl 36 IS subclones 1, 2, 7, 11, and 14, and cl 36 IS subclone 2 IS cl 4 and 7 contained heteropolymers of the human and mouse isozymes.
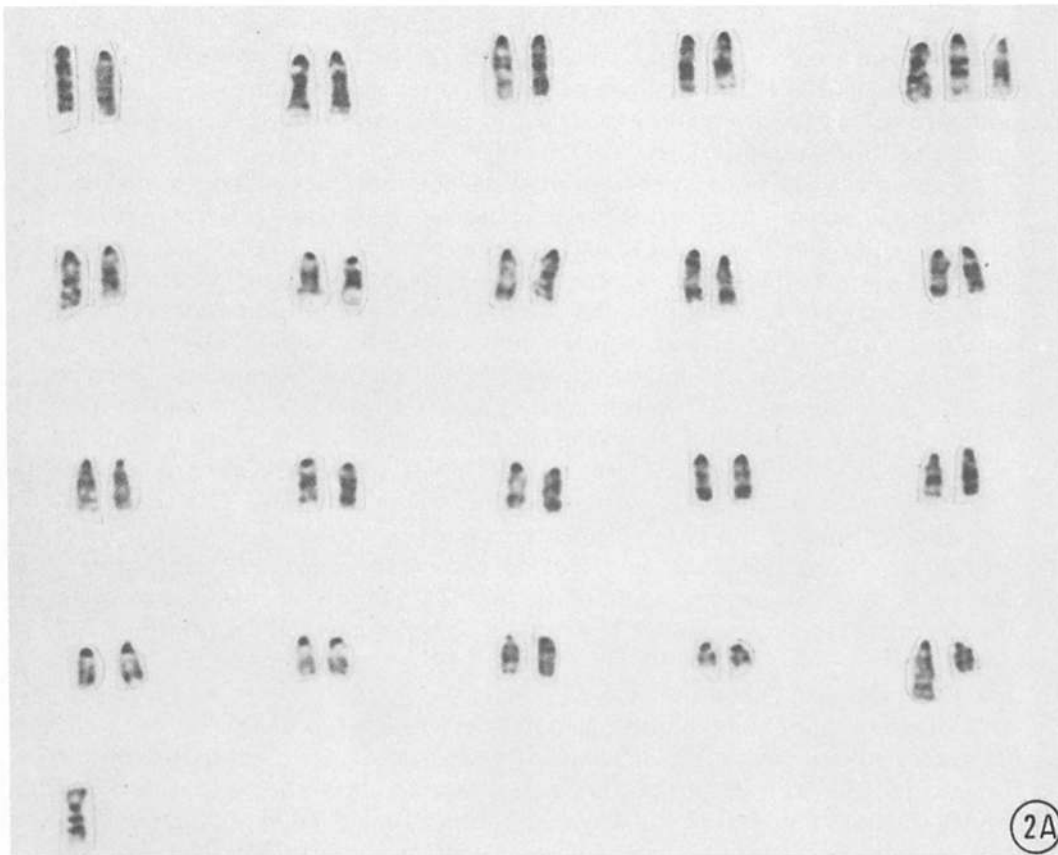
FIG. 2. A. Karyotype of cl 36 before immunoselection.

Discussion

These results further define the human cell surface antigen(s) coded for by human chromosome 7 (10). Antisera, generated in mice syngeneic to the mouse-human hybrid cell containing chromosome 7 from the LN-SV parent cell as the only human chromosome, maximally reacted in CDL with LN-SV and the hybrid clones 21 and 36 containing chromosome 7 from LN-SV. Positive cytotoxic reactions were observed on only two of the nine other primate cell lines tested. These results are in contrast with those obtained with indirect RIAs on the same cell lines in which there was significant binding to cells negative in CDL. It is possible that two or more cell surface antigens are coded for by genes located on human chromosome 7 derived from LN-SV and that only one of these antigens results in cytotoxic lysis of cells in the presence of antiserum and C. Thus, cells expressing this antigen are lysed; cells that do not express it are not susceptible to CDL but still bind antiserum due to the presence of the second antigen. Another possible explanation is that alleles at the same genetic locus segregate in the human population. LN-SV, HT1080, and RDMc would thus contain the same allele and the other primate cells tested would possess different alleles. The latter alleles could then code
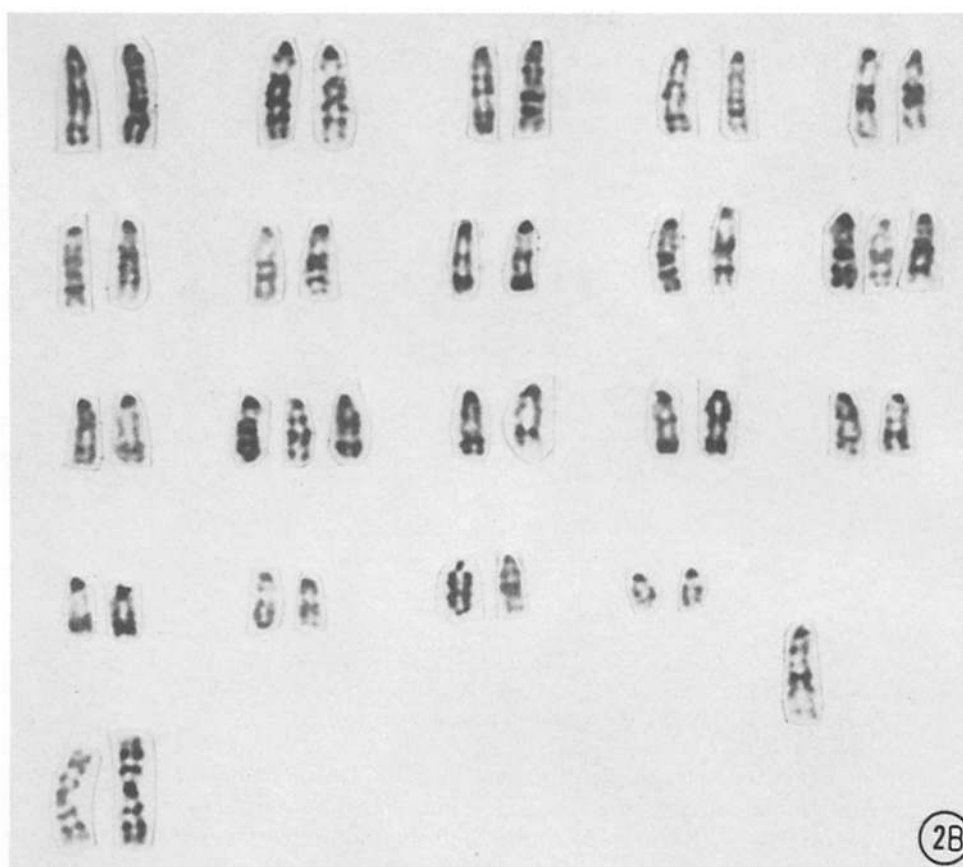
FIG. 2. B. Karyotype of cl 36 IS subclone 2.

for cell surface antigens capable of binding antiserum but incapable of antiserum and C binding in a manner necessary for cytotoxic lysis.

In confirmation of our previous data in RIA (10), anti-cl 21 serum appears to contain no reactivity (CDL) to SV40 TASA as evidenced by the negative results on SV40-transformed primate (W18Va2, 1054-TR, and GMK EVa cl 2A-1) and SV40-transformed rodent (F5-1, WIRL-SV, MKSBu-100, C57SV) cell lines. This despite the fact that cl 21 hybrid cells do express SV40 TASA as they act as effective immunizing cells (in C57BL/6 mice) generating cytotoxic T cells specific for SV40-transformed syngeneic cells (14). It appears that in C56BL/6J the response to the SV40 TASA is primarily a T-cell response.

Reaction of one clone of hybrid cells containing human chromosome 7 as the only human chromosome with anti-cl 21 serum and C resulted in greater than 99% cell death. Those cells escaping CDL were grown to confluence either as a mass population or as clonal isolates. When the mass population of cl 36 IS was karyotyped, it was found that a proportion of these cells still contained an intact chromosome 7 (Table II). Approximately half of the cl 36 IS cells were susceptible to C-dependent antiserum lysis (Fig. 3). Clones initially derived from this mixed population fall into two groups: those lacking the relevant
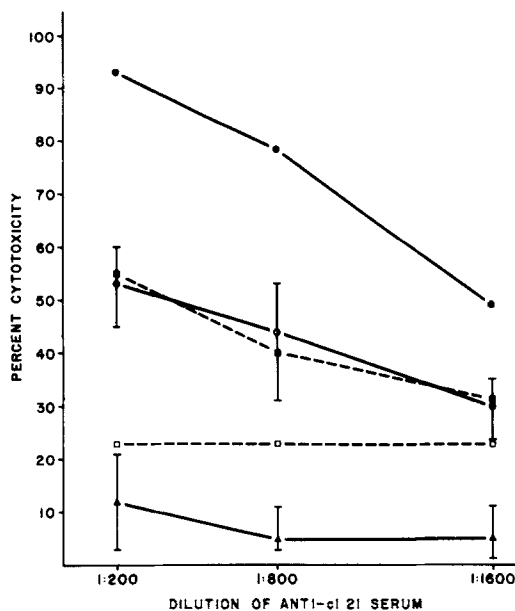
FIG. 3. Anti-cl 21 serum CDL tested on hybrid cl 36 cells before and after immunoselection: (●—●), cl 36; (■—■), cl 36 IS mass; (□---□), cl 36 IS subclone 2; (○—○), cl 36 IS subclones 3, 6, 8, and 11 (mean and range); (▲—▲), eight subclones of cl 36 IS subclone 2 and cl 36 IS subclones 1, 7, 9, and 14 (mean and range).

portion of the short arm of 7, nonreactive with antiserum and C; and cells containing this chromosome in a proportion of their metaphases and proportionately reactive with antiserum and C. Variable expression of the antigen throughout the cell cycle and inaccessibility of the cells to either antiserum or C because of position in cell aggregates are but two explanations for escape from specific lysis. Those cells containing rearranged chromosomes missing a portion of the short arm and those missing the entire short arm are no longer reactive with the antisera in CDL and bind only 20% as many counts as the parent human cell LN-SV in RIA. Absorption of anti-cl 21 serum with cells containing isoq7 did not reduce counts bound to LN-SV in RIA. The data from immunoselection experiments indicate that the antigen(s) responsible for CDL is coded for by a gene(s) located on the p arm of the human chromosome 7. This remaining reactivity in RIA might represent an additional minor antigen coded for by the long arm of the human chromosome 7 and this possibility is being tested by direct immunization with hybrids containing isoq7. It is possible that cryptic rearrangements of the short arm of human chromosome 7 into the mouse chromosomal material may have occurred in some or all of these hybrid clones. If translocations of this sort have occurred then a position effect for the normal expression of this antigen would have to be postulated.

Clones of cells missing the distal portion of the p arm of chromosome 7 and cells missing the entire p arm of human chromosome 7 retained the transformed phenotype associated with the integrated SV40 genome. SV40 T antigen was expressed in all cells of the IS hybrids and all of the clones caused tumors in nude mice. These data indicate that the SV40 genome is integrated in this hybrid clone either on the q arm of human chromosome 7 or in the
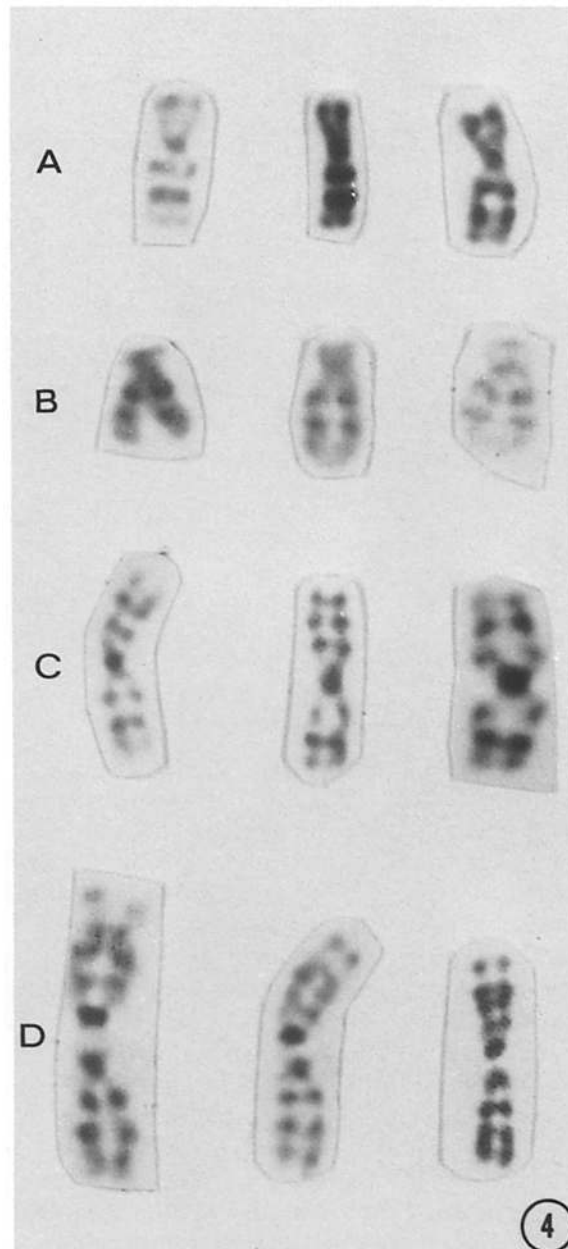
Fig. 4. (A) Human chromosomes 7 from three different metaphases of cl 36; (B) Human chromosome 7qKpl from three different metaphases of cl 36 IS subclones; (C) Human chromosome isoq7 from three different metaphases of cl 36 IS subclones; (D) Human-mouse chromosome 7 translocation (human 7qk7plT-Tk12 mouse) (see text) from three different metaphases of cl 36 IS subclones.

centromere region and further validate the findings of Croce et al. (7, 9) of mandatory retention of human chromosome 7 in hybrid cells prepared by fusing normal mouse macrophages with the SV40-transformed human cell line, LN-SV. The long arm of the human chromosome 7 was present in each of

TABLE II

Chromosome Constitution of Clone 36 and Immunoselected (IS) Derivatives of Clone 36

	Normal human 7*	Human‡ 7qter → 7pl	Human isoq7§	Rea (HSA + MMU) (7::12)‖	Metaphases analyzed n	Chromosome number mode (ranges)
	%	%	%	%		
cl 36	95		15	5	20	42 (38–44)
cl 36 IS mass	35	47	14	4	26	42 (40–43)
cl 36 IS						
Subclone 1				100	40	40 (38–41)
Subclone 3	12	33	9	56	23	40 (37–42)
Subclone 7				100	64	40 (38–41)
Subclone 9	10		100		10	40 (38–41)
Subclone 11	88	12	75		14	41 (40–43)
Subclone 14		23		77	43	40 (37–43)
Subclone 2		36	64		11	40 (39–41)
Subclone 2 IS						
Mass A		2	98		42	40 (!39–43)
Mass B			100		38	41 (38–43)
Mass C			100		20	41 (38–42)
Mass E						
Subclone 2				100	17	78 (74–81)
Subclone 3			68	32	32	41 (38–43)
Subclone 4				100	11	40 (38–79)
Subclone 5				100	5	41 (40–41)
Subclone 7			100		17	41 (39–42)
Subclone 8				100	11	40 (39–41)
Subclone 11			100		3	40
Subclone 12			100		8	40 (40–43)

* Arm ratio: short arm/total chromosome length (0.31–0.43), see Fig. 4 A.
§ See Fig. 4 C
‡ Arm ratio: short arm/total chromosome length (0.21–0.31), see Fig. 4 B.
‖ See Fig. 4 D.

over 400 metaphase spreads from 17 subclones and 4 mass populations of cl 36 subjected to immunoselective pressures against chromosome 7. The portion of chromosome 7 containing the SV40 genome is retained in the hybrid cells and presumedly the conservation of SV40 is required for growth of the mouse macrophage which as a normal cell does not divide. Barring translocation of the relevant portion of the long arm of the chromosome 7 for the SV40 genome to a mouse chromosome, the centromere of chromosome 7 must also be retained to ensure the segregation of the integrated SV40. Since immunoselection results in growth of only those cells containing rearranged chromosomes already present at the time of the selection event we can only hypothesize about the conditions leading to the presence of the three chromosome rearrangements in the original hybrid population before immunoselection. Assuming that breakage events along the entire chromosome 7 are equally probable and that retention of the SV40 integration site is obligatory, we should find some clones missing the intact long arm of chromosome 7. Long arm rearrangements should be found but in fact we only observed various short arm losses. This could indicate that either the short arm is more susceptible to breakage, or that long arm rearrangements did occur but were missed in the limited number of cells surveyed or that the centromere through q terminus of the human chromosome 7 is necessarily retained due to coadaptation of the SV40 integration site and some other gene(s) contained on chromosome 7. This point should be resolved by further investigation of hybrid clones of different origin.

Analysis for β-glucuronidase of clones containing each of the rearranged human chromosomes 7 indicated that both mouse and human enzyme forms and heteropolymers between them were expressed in these hybrids. This result is in agreement with the previous assignment of the human enzyme to chromosome 7 (13) and provides preliminary evidence that this gene might be located on the long arm of chromosome 7.

These experiments suggest that rearrangements of any chromosome retained by stringent selection in somatic cell hybrids may be obtained in hybrid cells providing cell surface antigens coded for by the same chromosome exists.

Summary

Immunoselection via complement-dependent lysis of human-mouse somatic cell hybrids containing chromosome 7, with antisera reactive to cell surface antigen(s) coded for by chromosome 7, has resulted in growth of somatic cell hybrids containing rearranged human chromosome 7s. Investigation of these hybrids has localized the gene(s) coding for the relevant cell surface antigen(s) to the short arm of human chromosome 7. The simian virus 40 integration site and the gene coding for human β-glucuronidase appear to be localized to the long arm of chromosome 7 in this hybrid clone.

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