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Immunoglobulin G and Free Kappa-Chain Synthesis in Different Clones of a Hybrid Cell Line

(BALB/c myeloma cells/C_{57} BL/6n lymphoma cells/azaguanine-resistant/bromodeoxyuridine-resistant)

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ABSTRACT 13 clonal cell lines were isolated from a hybrid cell population established by cell fusion between cloned BALB/c myeloma cells that were resistant to 8-azaguanine and produced immunoglobulin G and free kappa chain and C_{57}BL/6n lymphoma cells that were resistant to bromodeoxyuridine and did not produce immunoglobulins. Some of the histocompatibility-2 antigens of both parental cell lines could be demonstrated on all of the hybrid clones. 11 clones synthesized only free kappa chain. Two clones synthesized IgG of BALB/c type, as well as free kappa chain. These clones had higher chromosome number than did the clones that synthesized only kappa chains.

Cellular differentiation may be thought of as an orderly and tightly controlled process of selective gene expression. Recent experiments support this notion by demonstrating that large numbers of unexpressed genes are present in the nucleus of the differentiated cell (1–3). One approach to the study of the biochemical control mechanisms in cellular differentiation has been hybridization of cells that differ in their expression of specific genes. Such hybridization studies, however, have been complicated because of various degrees of heterogeneity in chromosome number present in the parental cell lines and also because of chromosome losses from the resulting hybrid cells upon successive cell divisions.

I have attempted to circumvent these difficulties by carefully choosing homogeneous parental cell lines for hybridization, and by subsequent cloning of the resulting hybrid cells. A hybrid cell line was established by cell fusion between a cloned BALB/c myeloma that produced immunoglobulin IgG and free kappa chain, and C_{57}BL/6n lymphoma that did not produce any immunoglobulins. The resulting hybrid cell population synthesized free kappa chain, but no synthesis of IgG (γ_{2a}) heavy chain was detected (4). I now report isolation of IgG (γ_{2a}) heavy-chain producers from these hybrid cells by a single-cell cloning technique. It will be shown that whether a hybrid cell is producing IgG (γ_{2a}) heavy chain and free kappa chain or only free kappa chain may be related to the chromosomal composition of the cell.

MATERIALS AND METHODS

Tissue Culture Conditions and Media. Cells were grown in Eagle's minimal essential (suspension) medium, supplemented with 400 mM glutamine, 1 ml of 100-times concentrated nonessential amino acids, and 1 ml of 100 mM sodium pyruvate (Microbiological Associates, Inc.); 100 units of penicillin and 20 ml of fetal calf serum per 100 ml of Eagle's medium (5).

Production of Hybrid Cells. Hybridization was performed as previously described by the use of Sendai virus inactivated with β-propionolactone. Growing hybrid cells were selected for in HAT medium (4).

Cloning Technique. Cloning was done by a modification of a technique described by Puck (6). Glass rings of 6-mm height and 7-mm internal diameter were cut from glass tubing. After thorough washing, they were soaked in saline at 37°C for a minimum of 30 min, rinsed three times with deionized–glass distilled water, and dried. Then, the rings were rimmed with nontoxic high-vacuum silicone grease (Dow Corning) and autoclaved. 10–30 cells of a monodisperse suspension in 3 ml of medium were added to 60-mm tissue culture dishes (Falcon plastics). After 2-hr incubation at 37°C in a CO_2 incubator, the attached single cells were visualized by phase microscopy and isolated within a glass ring. The silicone grease helped to seal off the isolated cell from the remainder of the cells in the plate. Cell division within the glass rings was followed each day and the medium within the ring was changed every 4 days. When the number of cells reached 500 or more within a ring, the cells were gently removed with a rubber policeman and subcultured. The decanted medium from each ring was tested for secreted immunoglobulins. To assure that there was no leakage in the silicone grease seal that might have allowed cells from outside the ring to enter into the cylinder, concentrated trypan blue dye or bacteria were added to the medium surrounding the glass ring 18 hr before subculturing. Dye or bacteria seldom entered a ring; most rings were perfectly sealed.

Other Procedures. Histocompatibility-2 (H-2) antigens present on the cells were determined according to Rogentine (7) by measurement of ^{51}Cr release from labeled cells in the presence of specific H-2 antisera (Table 2) and rabbit complement. Chromosome preparations were made by a modification of the method described by Moorheat *et al.* (8), and chromosome numbers for each cell line were determined by counting the chromosomes in photomicrographs of 25 randomly chosen metaphase cells. Immunoglobulins were determined by Ouchterlony gel diffusion and immunoelectrophoresis by use of monospecific antisera (4).

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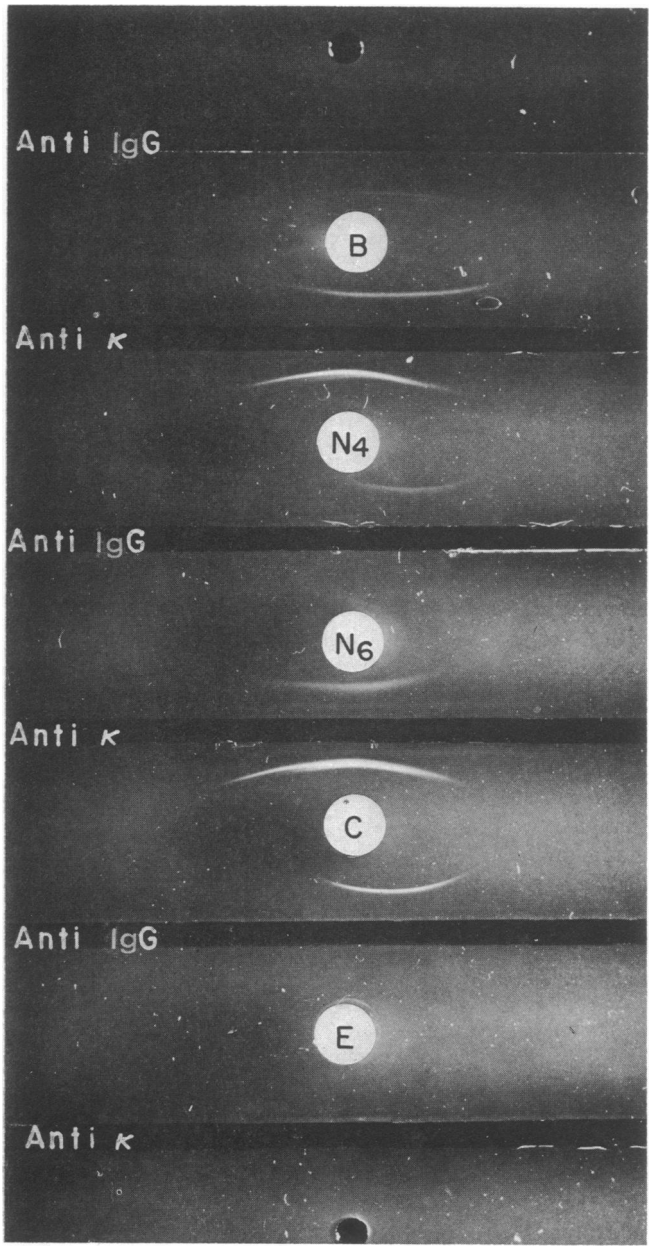

FIG. 1. Immunoelectrophoresis was done on proteins secreted by 2-day cultures of 1×10^7 cells concentrated to 0.2 ml (100 times) B = BALB/c serum; N_6 = N_6 hybrid; N_4 = N_4 hybrid; C = CL_4 (azaguanine-resistant) parent; E = EL_4 BrdU parent. Anti IgG and anti-kappa chain (anti κ) sera were monospecific for mouse IgG and kappa chains (4).

RESULTS

Characteristics of the parental cell lines and the mixed hybrid cell population

The BALB/c myeloma parent was established from a RPC-5 tumor generously provided by Dr. Michael Potter. It was subsequently cloned. One of the clones (CL_4) synthesized IgG and free kappa chain, and had a modal chromosome number of 61 that was stable in culture for 2.5 years. At this time, it was made resistant to 15.2 μg/ml of 8-azaguanine (4). The 8-azaguanine-resistant subline continued to synthesize IgG and free kappa chain and had a modal chromosome number of 60 (Table 1). It was used as one of the parents for hybridization.

The other parent was the bromodeoxyuridine-resistant EL_4 subline. This cell line was established from a lymphoma induced in a $C_{57}BL/6n$ mouse by 9,10-dimethyl-1,2-benzanthracene (9). It had a diploid modal chromosome number [40] with a narrow range, therefore, it was made resistant to 100 μg of bromodeoxyuridine per ml, without cloning. This bromodeoxyuridine-resistant subline had a modal chromosome number of 39 and did not produce any immunoglobulins (Table 1). The $EL_4 \times CL_4$ hybrid cell population had a wide range of chromosome numbers [70–103]. Only kappa chain could be detected in this hybrid population. No intracellular or secreted IgG was detected (ref. 4, Table 1).

Growth in mice

The 8-azaguanine-resistant myeloma cells could easily be grown in BALB/c mice by injecting 5×10^5 or more cells subcutaneously. It continued to produce IgG and free kappa chain in the mouse. The bromodeoxyuridine-resistant lymphoma cells would easily grow in $C_{57}BL/6n$ mice as subcutaneous or intraperitoneal tumors. The hybrid cell population did not grow in BALB/c or $C_{57}BL/6n$ mice when 1×10^7 cells were injected subcutaneously, but grew in BALB/c × $C_{57}BL/6n$ F_1 hybrids. Its growth rate was about one half that of the parental cell line. It produced Bence-Jones kappa chains that were excreted in the mouse urine, but no myeloma IgG was detected in the serum of the mice that bore tumors.

Characteristics of isolated clones of hybrids

Two groups of clones were isolated from the mixed hybrid cell population. Seven clones (M_1–M_7) were isolated from the hybrid cells that were grown in HAT medium for 3 months, passaged through mice for two generations, and subsequently maintained in HAT medium for 4 additional months. Six other clones (N_1–N_6) were isolated from the hybrid cells that were grown in HAT medium for 3 months and then frozen in liquid nitrogen for 5 months. M_1–M_7 and 4 of the N series of clones produced only free kappa chains. Clones N_3 and N_6, however, synthesized easily detectable amounts of IgG, as well as free kappa chains (Fig. 1, Table 1). The IgG heavy chains synthesized by these clones (kindly tested by Miss R. Lieberman) were of the BALB/c myeloma type, as shown by Ouchterlony gel diffusion with idiotypically specific antisera. Media from 2-day cultures of 1×10^7 cells of clones N_3 and N_6 were concentrated to 0.2 ml (100 times) and tested for the presence of $C_{57}BL/6n$-type IgG. The assay, kindly performed in Dr. L. A. Herzenberg's laboratory, consisted of inhibition of precipitation of radioactive $C_{57}BL/6n$ IgG by allospecific antiserum. This assay could have detected as little as 3 μg of $C_{57}BL/6n$ IgG per ml. None was detected. The same samples contained about 1 mg/ml of BALB/c-type IgG.

It was particularly interesting that clones N_3 and N_6, which synthesized both IgG and free kappa chains, had the highest mean chromosome-number. Marker chromosomes of both parental cell lines were found in all the hybrid clones.

H-2 antigens of both parental cell lines were demonstrated in IgG-producing as well as kappa-producing clones.

DISCUSSION

The data presented provide evidence that immunoglobulin synthesis is maintained in hybrid cells derived from cloned immunoglobulin-producing mouse myeloma cells and non-producing mouse lymphoma cells. Most of the hybrid clones

synthesize and secrete detectable quantities of kappa light chains only. Some clones, however, synthesize and secrete both IgG (γ_{2a}) and free kappa chain. The IgG heavy chain synthesized is of BALB/c (myeloma) specificity. No C_{57}-BL/6n (lymphoma)-type heavy chain is detected. Furthermore, those clones that synthesize both IgG and kappa chain have the highest mean chromosome number (Table 1).

TABLE 1. *Comparison of karyotypic composition and immunoglobulin synthesis of various clones isolated from the hybrid cell population*

Cell lines*	Chromosomes				Intracellular and secreted immunoglobulins	
	Mode	Mean	Range	Markers	IgG	Kappa chain
CL_4	61	63.0	61–67	2m or 3m	+	+
CL_4(azaguanine-resistant†)	60	61.0	60–64	2m or 3m‡	+	+
EL_4	40	40.5	39–42	1s§	−	−
EL_4-(bromodeoxyuridine-resistant)	39	39.5	38–41	1s	−	−
$EL_4 \times CL_4$† (mixed hybrid)	92	89.7	70–103	2m; 1s	−	+
M_3	88	89.1	90–86	2m; 1s	−	+
M_6	90	91.3	90–94	2m; 1s	−	+
M_5	90	91.4	90–94	2m; 1s	−	+
M_2	91	91.6	90–94	2m; 1s	−	+
M_1	94	91.8	89–94	3m; 1s	−	+
M_4	94	92.7	90–95	3m; 1s	−	+
M_7	93	93.5	91–98	4m; 1s	−	+
N_5	86	86.6	84–89	2m; 1s	−	+
N_4	90	90.7	89–93	2m	−	+
N_1	90	91.4	90–94	2s; 1m	−	+
N_2	95	92.7	86–96	2m	−	+
N_3	94	98.0	90–97	2m; 1s	+	+
N_6	100	100.7	99–103	2m; 1s	+	+

* CL_4 is the cloned BALB/c myeloma parent; EL_4 is the diploid C_{57}BL/6n lymphoma parent. M_1–M_7 are clones isolated from the hybrid cell population, grown in HAT for 3 months, passaged through mice twice, and maintained in HAT for four more months. N_1–N_6 are clones isolated from hybrid cells grown in HAT for 3 months and frozen in liquid nitrogen for 5 months.

† The modal chromosome numbers of CL_4 and azaguanine-resistant cells were given as 57 and 54, respectively; while the range for chromosomes of EL × CL_4 hybrid cells was given as 70–97 in a preliminary report (4). This underestimation of chromosome numbers was the result of errors of direct counting of chromosomes under the microscope. The numbers given here were obtained by counting the chromosomes in 25 photomicrographs of randomly selected metaphase cells. Secreted immunoglobulins were detected by Ouchterlony gel diffusion and immunoelectrophoresis of dialyzed and lyophilized medium from 1×10^7 cells. Intracellular immunoglobulins were determined similarly in lysates of 4×10^7 cells/ml. IgG present in clones N_3 and N_6 was precipitated with RPC-5 (i.e., BALB/c myeloma) idiotypically specific antisera.

‡ m = metacentric.

§ s = submetacentric.

TABLE 2. *Comparison of H-2 antigens of parental cell lines and hybrid clones*

Alloantiserum*	H-2 specificity	CL_4 (Azaguanine-resistant) parent	EL_4 (Bromodeoxyuridine-resistant) parent	Hybrid-clones N_1–N_6	Hybrid-clones M_1–M_7
C_2	2(C_{57}BL/6n	0†	+‡	+	+
C_8	8,9(BALB/c)	+	0	+	+

* The antisera were obtained from Dr. George Snell (PH 66–483), under contract with Transplantation and Immunology Branch, NIAID, NIH, Bethesda, Md. The nomenclature of antisera and their specificity are taken from the catalog of mouse alloantisera compiled by George Snell, 1968.

† 0 = antigen absent.

‡ + = antigen present (lysis at least 15% higher than control).

§ See Table 1, legend.

These observations may be interpreted as follows: (*a*) IgG heavy-chain or kappa light-chain synthesis in these hybrid cells may be directly attributable to the presence in the cells of the myeloma chromosome(s) that carry the gene(s) for these polypeptide chains; (*b*) in the mouse, the genes for IgG heavy chain and kappa light chain are probably located on different chromosomes. This assertion is consistent with the observation that the hybrid clones that synthesize both IgG and kappa have higher chromosome numbers than those that synthesize only kappa chain. It is known that, in the rabbit, the genes for light and heavy chains of immunoglobulins are not linked (10, 11); (*c*) synthesis of IgG heavy chain in the hybrid cell is not the simple consequence of the presence of "gene activators" or other positive-control factors contributed by the CL_4-8-azaguanine-resistant parent. If this were true, one would have expected the silent IgG genes of C_{57}BL/6n type, which were presumably present in the IgG-synthesizing hybrid cells, to have been activated. However, no C_{57}BL/6n-type IgG heavy chain was detected in such hybrids; (*d*) detection of IgG heavy chain in hybrids with the highest chromosome number provides a strong argument against the notion that gene repressors or other negative-control molecules could have caused the absence of immunoglobulin synthesis in an EL_4 (bromodeoxyuridine-resistant) parent. If such negative-control factors were operative in this cell line, one would have expected both IgG synthesis and kappa light-chain synthesis to stop in hybrids with the highest chromosome number. Such hybrids, presumably, would have contained the EL_4 (bromodeoxyuridine-resistant) cell chromosomes for the "repressors".

These conclusions, however, may be challenged by several arguments.

(i) It was shown that the EL_4 (bromodeoxyuridine-resistant) parent had one chromosome less than the diploid number for the mouse. It is conceivable that the genes for both kappa light chain and IgG heavy chain, as well as the "repressor" genes for these polypeptides, were on that one chromosome. This possibility, in my view, is unlikely.

(ii) Specific immunoglobulin gene "repressors" or "activators" may, indeed, exist but show allotypic specificity. Then, it would not be surprising that their presence could not be demonstrated in hybrids derived from cells of two different allotypic backgrounds. This argument derives further strength when it is recalled that the individual immunoglobulin-producing cells in a heterozygous animal synthesize immunoglobulin of one allotype only, a phenomenon known as "allelic exclusion" (12,13). In this regard, it is of interest to recall that hybrid clones of a cross between myeloma MOPC 315 of BALB/c origin that produced IgA and fibroblasts CL1D of a C_3H/He mouse produced little or no immunoglobulin (14). This is entirely expected if repressors for immunoglobulin synthesis require allotypic specificity, because it is known that IgA of BALB/c and C_3H/He mice are of the same allotype (15). The answer to this argument must await the results of studies on hybrids between two different immunoglobulin-producing myelomas of BALB/c mice. The interpretation of such studies, however, may be complicated by similar arguments invoking this time the idiotypic specificity of the control molecules involved in gene expression.

(iii) Finally, lack of repression of immunoglobulin synthesis in the present hybrid cells may reflect a mutation(s) at the "operator-gene" region of the immunoglobulin operon of CL_4-8-azaguanine-resistant myeloma parent. Such a mutation could have rendered the immunoglobulin operator gene(s) of the myeloma incapable of perfect binding of functional repressor that may have been contributed to the hybrid cells by the nonproducing EL_4 bromodeoxyuridine-resistant parent. Examples of "operator gene" mutants incapable of binding normal functional repressor are well documented for the *lac*-operon of *Escherichia coli* (16). Such mutants synthesize large amounts of *lac*-operon gene products in the presence of normal repressor (17). The possibility of occurrence of such an "operator-gene" mutation in a functional tumor such as CL_4-8 azaguanine-resistant myeloma may not be altogether remote. It is known that cell division and immunoglobulin synthesis are closely linked functions in normal lymphoid cells (18, 19). Therefore, if it is assumed that the processes of cell division and immunoglobulin synthesis in normal lymphoid cells are controlled by the same "operator gene", a single mutation at this gene locus could account for the emergence of immunoglobulin-synthesizing tumors. Elucidation of this intriguing possibility must await the development of inducible immunoglobulin-producing cell lines or the isolation of the hypothetical immunoglobulin gene repressors. In any event, it is difficult to imagine that "operator gene" mutations could account for the orderly process of selective gene expression seen in cellular differentiation, even though such mutations may be the basis of the emergence of differentiated tumors such as the myeloma.

Several examples of loss of differentiated function in somatic hybrids have been reported. These include extinction of melanin synthesis in melanoma-fibroblast hybrids (20) and of secretion of growth hormone in pituitary–fibroblast hybrids (21). There are also examples of differentiated functions such as synthesis of hyaluronic acid and collagen that have continued when producer and nonproducer cells were hybridized (22). It is possible to explain the expression or lack of expression of a specialized gene in these hybrids by either the presence or absence of the chromosome carrying the structural gene or the gene specifying the repressor. In addition, various "operator genes" may have different degrees of specificity requirements for interacting with repressors. For example, the specificity requirement for immunoglobulin genes may be more stringent. Notwithstanding these arguments, an important point emerging from the present investigation is the need for caution in attributing gene "turn on" or "turn off", and thus deducing the presence of hypothetical control molecules in a mixed hybrid-cell population derived from heterogeneous parental cell lines. Closer attention to the karyotypic composition and homogeneity of the parental and hybrid cells to be studied are needed before conclusions regarding cellular control mechanisms are drawn.

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