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Immunology

Human hybridomas constructed with antigen-specific Epstein–Barr virus-transformed cell lines

(ouabain resistance/thioguanine resistance)

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ABSTRACT **A 6-thioguanine-resistant, human lymphoblastoid B-cell line (GM1500 6TG A-11; IgG secreting) was mutagen-treated with low-level γ-irradiation and selected for ouabain resistance. One line showing 10,000-fold higher drug resistance, designated KR-4, was fused with an Epstein–Barr virus-transformed, cloned, B-lymphocyte cell line (B6) producing antitetanus toxoid (TT) antibody (IgM), and the hybrids were selected in hypoxanthine/aminopterin/thymidine medium containing 10 μM ouabain. Surviving cells, which arose at an optimal frequency of 10^{-5}, were subcloned by limiting dilution and screened for anti-TT production. Out of 395 final subclones, 372 were found positive for anti-TT, and seven that were selected for further study secreted specific antibody (IgM, κ chain) at a maximum concentration of 3–6 μg/ml. The differential rate of anti-TT production during the logarithmic phase of cell growth was 15-fold higher in the hybridomas than in the original B6 line. The hybrid nature of the clones was confirmed by karyotype analysis, histocompatibility antigen typing, and expression of secreted and membrane-bound Ig classes. Biosynthetic labeling of the cells revealed that all hybrids secreted both IgM and IgG but that only the IgM class had specificity for TT. Because Epstein–Barr virus is a polyclonal B-lymphocyte activator, the technique we applied here may be useful for increasing the recovery of rare antigen-specific B cells in the peripheral blood and for improving the frequency and stability of hybridomas secreting a given antibody.**

The production of human monoclonal antibodies expressing a desired specificity has proved successful with methods that use either the lymphotropic Epstein–Barr virus (EBV) to immortalize antigen-specific B cells (1–5) or the classical hybridoma technique, whereby human blood lymphocytes are fused with murine (6) or human myeloma cell lines (7, 8). To overcome difficulties inherent in each technique (9), we examined the possibility of combining them. We have described a technique whereby lymphocytes from normal donors immunized with tetanus toxoid (TT) were preselected for antigen-binding cells, subsequently transformed with EBV, and cloned (10). In the present study, an EBV-transformed clone B6 was fused with a human lymphoblastoid cell line of the B-cell type (B-LCL), KR-4, to rescue high amounts of anti-TT antibody production. The resulting hybridomas were found to be more stable, to have a higher cloning efficiency, and to secrete ≈8-fold more specific antibody compared with the parental B6 clone. In addition KR-4 had a 25-fold higher frequency of hybridization with EBV-transformed B cells compared with nontransformed B cells.

MATERIALS AND METHODS

Cell Lines. B6 is an anti-TT antibody-producing, EBV-transformed, cloned cell line that was previously established in our laboratory (10). GM1500 6TG A-11 is a 6-thioguanine-resistant ($SGua^R$) [hypoxanthine/aminopterin/thymidine (HAT)-sensitive] human B-cell line, which was generously provided by H. Koprowski at The Wistar Institute in Philadelphia (8). Both GM1500 and the KR-4 line that we derived were strongly positive for EBV nuclear antigen and both expressed surface-bound and cytoplasmic Igs as determined by immunofluorescence. Therefore, these lines are referred to as B-LCLs. Electron micrographs were consistent with this designation and revealed a less differentiated state than seen in myelomas.

Selection of an Ouabain-Resistant (Oua^R) B-LCL. The human B-LCL GM1500 6TG A-11 (10^6 cells per ml) was mutagen-treated with either ethyl methanesulfonate (60–150 μg/ml; Sigma) for 24 hr, or with γ-irradiation (100–300 R) (11). After mutagenic treatment, cells were allowed a 10-day period of expression in normal medium and then were seeded (2×10^6 cells per ml) in 96-well microtiter plates (Linbro) in 0.1-ml volumes in the presence of 0.1 μM ouabain (Sigma). Cultures were fed every 4 days with ouabain-containing medium, and wells with viable colonies were scored after 2 wk. The surviving cells were then subcultured in 24-well tissue culture plates (Costar) and subsequently grown in flasks (Falcon) in gradually increasing concentrations of ouabain. Dead cells were removed by the Ficoll-Isopaque method. The resulting line, KR-4, was found to be resistant to 0.5 mM ouabain 5 mo after mutagenesis.

Cell Fusion and Hybrid Selection. Prior to fusion, the $SGua^R$, Oua^R human B-LCL KR-4 was grown in the same medium as cryopreserved B6, supplemented with 30 μg of 6-thioguanine per ml and 0.1 mM ouabain to counterselect possible revertants. For fusion, 10^7 B6 cells and 10^7 KR-4 cells were washed in serum-free medium and then mixed in 50-ml tubes (Falcon). After centrifugation at $150 \times g$ at room temperature, cells were fused essentially as described by Köhler and Milstein (12) by using 45% (wt/vol) polyethylene glycol (M_r, 4,000; Sigma) in RPMI medium (pH 7.4). Fused cells were washed and cultured in 96-well microtiter plates at 2×10^5 cells per well on 3,000 R-irradiated mouse spleen cells (0.5×10^6 cells per well) or unirradiated mouse peritoneal cells (5×10^3 cells per well) as feeders. Cells were cultured sequentially in HAT medium (Flow Laboratories, Mississauga, ON, Canada) containing 5 μM ouabain on days 1–3, HAT medium containing 10 μM ouabain on days 3–14, 100 μM hypoxanthine/16 μM thymidine on days 14–21, and finally RPMI 1640 medium containing 20% fetal calf serum. Control cultures of each parental cell line (B6 and KR-4) contained no surviving cells after 12 days in HAT medium plus ouabain. The first putative hybridoma cells could be detected ≈12 days after fusion.

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Abbreviations: B-LCL, lymphoblastoid cell line of the B-cell type; EBV, Epstein–Barr virus; HAT, hypoxanthine/aminopterin/thymidine; Oua^R, ouabain resistant; Oua^S, ouabain sensitive; $SGua^R$, 6-thioguanine resistant; TT, tetanus toxoid; ELISA, enzyme-linked immunoadsorbent assay; HLA, human histocompatibility antigen; L chain, light chain; H chain, heavy chain.

Chromosome Analysis. Cells in the exponential phase of growth were stained for chromosomes by the method of Worton and Duff (13). A minimum of 40 individual metaphases were recorded for each individual cell line.

Anti-TT Standard. The Ig fraction of serum from TT-boosted human donors was purified by affinity chromatography as described (10). Electrophoretically pure, preservative-free TT (lot AS1070) was obtained from Connaught Laboratories, Willowdale, ON, Canada.

Enzyme-Linked Immunoadsorbent Assay (ELISA). Supernatants from cultures (cell density, 10^6 cells per ml) were tested for anti-TT antibody by the ELISA technique with TT in the solid phase as described (10). For detection of class-specific Ig, microtiter wells were coated with 10 μg of the $F(ab')_2$ fragment of goat anti-human Ig per ml (Cappel Laboratories, Cochranville, PA), and antibodies that bound were detected with heavy (H) chain-specific, goat anti-human IgG or anti-IgM conjugated to alkaline phosphatase (Sigma).

Surface Ig and Human Histocompatibility Antigen (HLA) Typing. Cells were stained by immunofluorescence with class specific reagents as described (10). The HLA-A and -B antigens were assayed by the Immunology Laboratory of the Kingston General Hospital by a microcytotoxicity method as described by Terasaki and McClelland (14).

Cloning. Cloning was performed by limiting dilution (0.3 cells per well) in 96-well microtiter plates (Linbro) on feeder layers consisting of irradiated (3,000 R) mouse spleen cells (5 $\times$ 10^5 cells per well). Clones were fed by replacing the medium once a week. Positive wells were screened by ELISA for anti-TT antibody present in the culture supernatants.

Biosynthetic Labeling of Igs. Cells (density, 2 $\times$ 10^6 cells per ml) were cultured for 12 hr (viability 70–75%) and 24 hr (viability 20–30%) in methionine-free medium containing 15% dialyzed fetal calf serum, 50 μM 2-mercaptoethanol, and 50 μCi (1 Ci = 3.7 $\times$ 10^{10} becquerels) of [^{35}S]methionine per ml (1,004.9 Ci/mmol; New England Nuclear). Cells were pelleted and Igs from the tissue culture supernatants were precipitated with rabbit anti-human IgG, IgA, and IgM (Dako; Cedarlane Laboratories, Hornby, ON, Canada) by the *Staphylococcus aureus* technique (15) modified as described (16). The immune complexes were resuspended in Laemmli buffer, analyzed on 12.5% $NaDodSO_4$/polyacrylamide gels (17), and subjected to fluorography for 4 days at −70°C. Chromatographically purified human IgM and IgG (Cappel Laboratories) were used as markers.

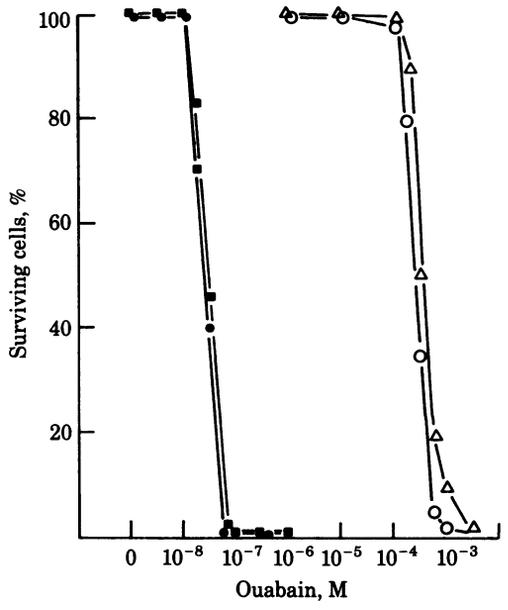

FIG. 1. Ouabain sensitivity of B6, Oua^S, and Oua^R B-LCL cells. Triplicate samples of 2 $\times$ 10^5 cells were seeded in 24-well Costar plates containing ouabain at concentrations ranging from 10 nM to 5 mM and in control wells without drug. Viable cells were counted after 6 days of culture by trypan blue exclusion. Survival was expressed as the percentage of control growth without drug. Oua^R KR-4 cells were grown 4 wk in the presence (△) or absence (○) of 100 μM ouabain. ●, Oua^S parental GM1500 6TG A-11 cells; ■, Oua^S EBV-transformed B6 line.

RESULTS

Selection of Oua^R Variants of GM1500 6TG A-11. The human B-LCL GM1500 6TG A-11 is deficient for hypoxanthine phosphoribosyltransferase activity (EC 2.4.2.8) as a result of a selection for SGua^R previously performed by Croce *et al.* (8). Cells were mutagen-treated by using low levels of γ-irradiation (100–300 R) or ethyl methanesulfonate (60–150 μg/ml) and selected for Oua^R. The highest frequency of Oua^R mutants (1 $\times$ 10^{-6}) was obtained after a 200-R irradiation treatment and was 5 times higher than the Oua^R frequency in untreated controls (2 $\times$ 10^{-7}) (Table 1, experiments 2 and 5). Exposure of the cells to ethyl methanesulfonate up to 150 μg/ml for 24 hr, the highest concentration tested, did not result in enrichment for Oua^R variants (Table 1, experiment 4) and might be due to the

Table 1. Selection of Oua^R cell lines and hybrids

Exp.	Mutagenic treatment*	Frequency of Oua^R mutants $\times$ 10^7,† mean ± SEM	Selected Oua^R cell line	Frequency of hybrid formation $\times$ 10^6,‡ mean ± SEM
1	100 R	8.1 ± 2.6	KR-3	3.4 ± 1.0
2	200 R	10.7 ± 4.8	KR-4	11.2 ± 2.1
3	300 R	7.2 ± 3.2	KR-2	0.14 ± 0.1
4	EMS, 150 μg/ml	2.4 ± 0.7	KR-5	0.20 ± 0.1
5	None	2.0 ± 1.5	KR-1	2.7 ± 0.7

* GM1500 6TG A-11 cells were mutagen-treated with either low γ-irradiation at the dose indicated or ethyl methanesulfonic acid (EMS) for 24 hr. After 10 days of growth in normal medium, cells were distributed in 96-well plates at 2 $\times$ 10^5 cells per well in the presence of 100 nM ouabain. Viable colonies were scored after 2 wk.

† Frequency was calculated from the fraction of negative wells with the Poisson equation. Mean frequency is given for four plates.

‡ Each cell line was fused with the B6 cell line. Viable colonies surviving the HAT/ouabain-selection procedure were scored after 12 days. Frequency was calculated from the fraction of negative wells (in one plate) by using the Poisson equation. Mean frequency is given for three plates, each derived from a separate fusion.

natural resistance of the GM1500 cells to this alkylating agent—a phenomenon occasionally encountered with permanent tumor cell lines. Mutant cells were grown in gradually increasing concentrations of the drug and, after 5 mo, one of them (KR-4) was found to be resistant up to 500 μM ouabain (Fig. 1). Because we did not test the level of resistance at the end of each successive step, it is not clear whether the mutants selected initially were already highly resistant to the drug or not. The extent of multiplication of ouabain-sensitive (Oua^S) myeloma cells and EBV-transformed B6 cells was reduced to half that of drug-free controls at 40–50 nM ouabain, whereas it was half reduced at 500 μM for the Oua^R myeloma mutant called KR-4. Therefore, KR-4 appears to be 10,000-fold more resistant to ouabain than is the B6 clone. The stability of Oua^R inheritance is evidenced by the similarity of the dose–response curves obtained with KR-4 cells grown in drug-free medium for 4 wk or maintained continuously in 100 μM ouabain (Fig. 1).

Selection of Hybrid Cells. One Oua^R B-LCL isolated from each of the five experiments in Table 1 was fused with the EBV-transformed B6 clone in the presence of polyethylene glycol, and hybrids were selected in the HAT medium formulated by Littlefield (18) supplemented with 10 μM ouabain. This intermediary concentration of ouabain was chosen because Oua^R is known to behave as a codominant trait in somatic cell hybrids (19). The frequency of hybrid formation between different variants (KR-1 to KR-5) and B6 was estimated 12 days after selection was initiated by scoring the fraction of negative wells and applying Poisson's equation. The highest hybridization frequency (1×10^{-5}) was obtained with the KR-4 cell line in several repeat experiments (Table 1). No clone arose from control plates containing each of the parental cell lines grown in the same selective medium. In parallel experiments, fusion of KR-4 with fresh nontransformed B cells resulted in a much lower (96%) hybridization frequency of $4 \pm 0.2 \times 10^{-7}$. Therefore, KR-4 cells may fuse preferentially with EBV-transformed cells compared to nontransformed cells.

Characterization of Hybrids. Fifty-six hybrids between KR-4 and B6 were expanded, and four of them were subcloned by limiting dilution. The parental B6 cell line has a modal chromosome number of 46 (range 37–51) similar to that of the KR-4 line (range 40–50) (Table 2). Approximately 3–4% of the metaphases in both cell lines had a near-tetraploid karyotype in the range of 84–91 chromosomes. Nine independent hybrids and 12 independent subclones obtained by limiting dilution were examined 2 mo after isolation for chromosomal content. Although some variation was noticed, due to the chromosome number scattering of each parental cell line, all hybrids were near tetraploid (range 74–99), attesting that only moderate chromosomal segregation took place. The best examples are HYB-20.42 and HYB-10.3, which had a mode of 91 chromosomes—very close to the expected tetraploid number of 92 (Table 2). No clear chromosome number difference was found between secretor and nonsecretor hybrid subclones. These results are comparable to the karyotype analysis reported by Clements *et al.* (20) on somatic cell hybrids between established human lymphoid cell lines carrying or not carrying the Epstein–Barr virus.

Further confirmation of the hybrid nature of the clones came

Table 2. Characterization of hybrid and parental cells

Trait	B6	KR-4	B6–KR-4 hybrids		
			Clone 20.42	Clone 10.3	Clone 8.10
Modal chromosome no.*	46 (37–51)	46 (40–50)	91 (79–96)	91 (83–99)	86 (74–94)
% cells in the mode	39	52	28	23	16
Surface Ig isotype†					
Total Ig	+	+	+	+	+
IgM	+	−	+	+	+
IgG	−	+	+	+	+
IgA	−	−	−	−	−
κ	+	+	+	+	+
λ	−	−	−	−	−
Total secreted Ig‡					
IgM	+	−	+	+	+
IgG	−	+	+	+	+
Anti-TT titer§	0.7	0.0	3.0	5.7	4.1
Mean rate of anti-TT production¶	0.39	0.00	5.33	5.71	6.87
HLA‖					
A2	+	+	+	+	+
A11	−	+	+	+	+
B8	+	−	+	+	+
B14	−	+	+	+	+
Bw4	−	+	+	+	+
Cloning efficiency, %**	30	ND	73	53	65

ND, not determined.

* Range is shown in parentheses. A minimum of 40 metaphase spreads were counted for each cell line.

† The isotype of surface Ig was determined by immunofluorescence using class-specific anti-Ig reagents. In general, all hybrids and KR-4 exhibited weak immunofluorescence (Leitz UV microscope), whereas B6 was strongly fluorescent.

‡ Total secreted Ig was determined in an ELISA assay with $F(ab')_2$ goat anti-human Ig in the solid phase.

§ Shown in μg/ml. Specific antibody was determined in an ELISA with TT in the solid phase. Cells were at the steady state or declining phase of growth (day 9).

¶ Shown in μg per 10^5 cells, determined from experiments described in Fig. 3 during logarithmic phase of growth.

‖ HLA haplotypes were determined by antibody and complement cytolysis in Terasaki plates.

** Cloning was performed by limiting dilution on feeder layers and inoculating at 0.3 cell per well.

from an analysis of HLA antigens expressed on parental cells and hybridomas. As expected, several HLA haplotypes were codominantly expressed in the hybrids (Table 2). In addition, KR-4 was found to secrete IgG and B6 secreted IgM exclusively whereas KR-4–B6 hybrids produced both IgM and IgG, in agreement with the class of Ig detected on the cell surfaces by immunofluorescence (Table 2).

Proteins produced by each cell line were biosynthetically labeled with [^{35}S]methionine, and the Igs were precipitated with class-specific rabbit anti-human Ig and *Staphylococcus aureus* protein. Precipitates were electrophoresed in 12.5% Na-DodSO$_4$/polyacrylamide gels, and, as shown in Fig. 2, a representative KR-4–B6 hybrid clone secreted high amounts of both μ and γ chains from each parent, as well as light (L) chain after 24 hr. The KR-4 parent secreted low levels of γ and L chains, whereas the B6 parent secreted a large amount of μ chain and little L chain. The coexpression of Ig H and L chains characteristic of each parental cell line further substantiates the hybrid nature of the selected fusion products.

Antibody Production. Fifty-four out of 56 hybrids between KR-4 and B6 were positive when screened by ELISA for production of anti-TT antibody. The four most positive hybrids were subsequently cloned by limiting dilution. Out of 395 subclones, 23 did not produce detectable amounts of anti-TT antibodies, the majority produced varying levels, and seven secreted high levels (3–6 μg/ml) of IgM κ chain anti-TT antibody. The mean cloning efficiency of three hybrids (64%) was at least 2-fold higher than the cloning efficiency of the B6 line (Table 2).

Three of these hybridoma clones selected for further studies produced 3, 4, and 6 μg per ml, respectively, of anti-TT antibody at the end of a growth period of 9 days (Fig. 3), starting from an inoculum of 2×10^4 cells per ml. The B6 parent produced 75–87.5% less anti-TT antibody and KR-4 produced none (level of detection, 1 ng/ml). The rate of antibody production during the logarithmic phase of cell proliferation was 13- to 18-fold higher in the hybrids than in the B6 parental line when a mode of representation described by Monod *et al.* was used (21). The rate of antibody production was not constant but exponentially increased for all cell lines tested (Fig. 3). This is not unexpected because production of antibody in the supernatant is the terminal stage of a sequential process that involves synthesis, assembly, and secretion, all of them being programmed and coordinated during the cell cycle. The B6 parent was unusual in that higher titers were detected during the declining phase of cell growth, possibly due to the release of intracellular and membrane Ig from dying cells.

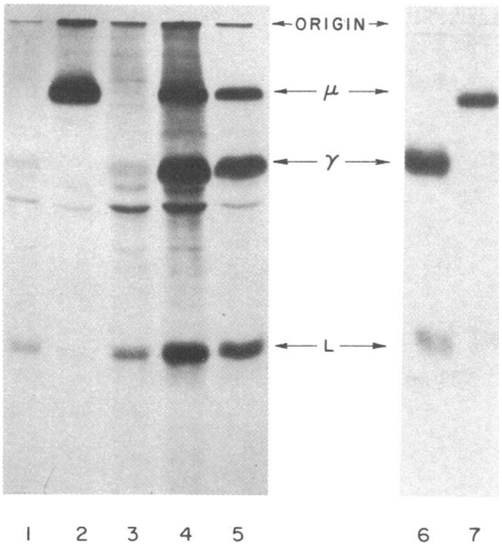

FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoresis of immunoprecipitated Ig chains synthesized by hybrid and parental lines. Cells were cultured at a density of 2×10^6 cells per ml for 12 hr and 24 hr in methionine-free medium in the presence of [^{35}S]methionine (50 μCi/ml). The human Ig chains were precipitated with rabbit anti-human IgG and anti-IgM by the *Staphylococcus aureus* technique. Aliquots of immunoprecipitates were reduced and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis on a 12.5% polyacrylamide slab gel. Gels were developed by fluorography. Lanes: 1, KR-4 (12-hr pulse); 2, B6 (12-hr pulse); 3, KR-4 (24-hr pulse); 4, KR-4–B6 (24-hr pulse); 5, KR-4–B6 (12-hr pulse). Standards consisted of chromatographically purified human serum IgG (lane 6) and myeloma-derived IgM (lane 7) stained with Coomassie blue.

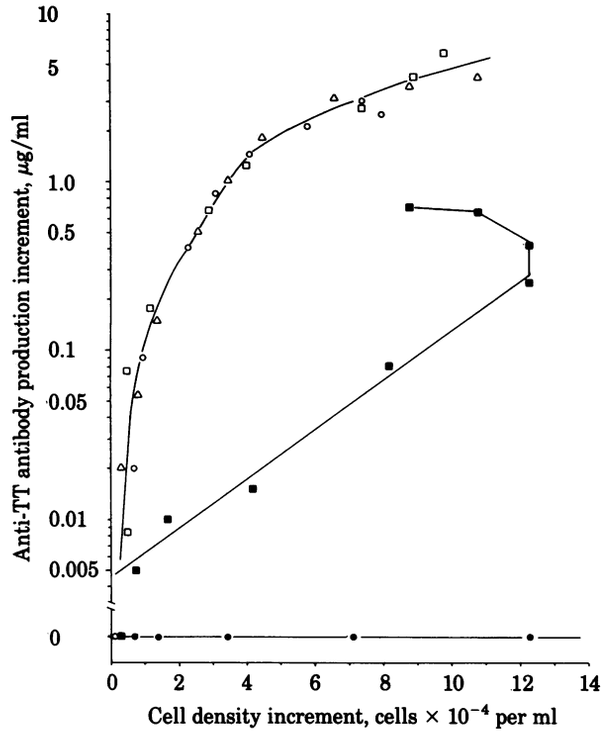

FIG. 3. Semilogarithmic-linear plot of the anti-TT antibody production increment versus the cell density increment. Cells at 2×10^4 per ml were seeded in 2-ml wells and grown for 9 days. Viable cells were counted daily and culture supernatants were measured for anti-TT antibody production by using an ELISA system with an immunoaffinity-purified anti-TT antibody as a standard. The increments were calculated by subtracting the initial antibody concentration or the initial cell density from the corresponding values measured each day, following the representation of Monod *et al.* (21). ●, KR-4 parent; ■, B6 parent; ○, hybridoma 20.42; □, hybridoma 8.10; △, hybridoma 10.3.

Our original EBV-transformed line, B6, produced ≈700 ng of anti-TT antibody per ml during continuous culture for 8 mo. When assayed again after 10 mo of culture, the level of anti-TT production had fallen to <10 ng/ml possibly because of the declining L chain synthesis, which was apparent at 8 mo of culture (Fig. 3). Fusion of KR-4 with B6 from 8-mo cultures "rescued" anti-TT production, and three clones designated 20.42, 8.10, and 10.3 maintained constant levels of anti-TT production (3,000–6,000 ng/ml) throughout 7 mo of continuous culture to the present time.

DISCUSSION

The results presented here demonstrate that relatively high amounts of specific monoclonal antibody can be rescued from

EBV-transformed B cells by fusion with a drug-resistant human B-cell line.

In order to allow adequate selection of hybrids, it was necessary to introduce an Oua^R marker into an already established $SGua^R$ B-LCL because the EBV-infected lymphocytes used for fusion are already immortalized and could not be counterselected otherwise. $SGua^R$ B-LCL cells were mutated by low-level γ-irradiation, selected for Oua^R, and fused with an EBV-transformed cell line (B6) that itself produced only small amounts of anti-TT antibody. Because of the dominance of the Oua^R marker and the recessiveness of the $SGua^R$ marker in the parental B-LCL (KR-4), only hybrid cells derived from the fusion of KR-4 and B6 could survive selection in HAT medium containing ouabain. The hybrid nature of the clones was confirmed by karyotype analysis, HLA typing, and Ig isotype expression. The hybridomas produced 4- to 8-fold more anti-TT antibody per 10^6 cells than the B6 parent did, whereas the rate of antibody synthesis and secretion was 13- to 18-fold higher in the hybrids. Biosynthetic labeling of proteins followed by $NaDodSO_4$/polyacrylamide electrophoresis of immunoprecipitates revealed that hybrids synthesized both μ and γ chains from the parental lines as well as κ L chains. The EBV-transformed B6 parental line synthesized very little L chain in comparison to μ chain, a finding that may explain the declining anti-TT titer observed during prolonged proliferation. It is noteworthy that anti-TT production in hybridomas remained stable for 7 mo up to the present time, whereas anti-TT production in nonfused B6 cells ceased after 10 mo of continuous culture.

The findings here support the experimental data of Levy and Dilley (6), who demonstrated that human neoplastic B cells, which do not normally secrete Ig, can be induced to secrete large amounts of Ig, when hybridized to a mouse myeloma line. Others have shown human Ig secretion by hybrids between mouse myeloma and normal human peripheral blood lymphocytes (22). We have fused an EBV-transformed cell line with a non-Ig-secreting mouse myeloma (23), and the established hybridoma clones produced anti-TT antibody. It is known, however, that human chromosomes 14 (H chain) and 22 (λ L chain) are preferentially retained in mouse–human hybrids, whereas chromosome 2 (κ L chain) is preferentially lost (24, 25). For this reason, human–human hybrids are to be preferred because intraspecific crosses are more stable. It is remarkable that, in our initial study, only 3.5% of the clones were found to be nonsecretors. The karyotype analysis also indicated that chromosome secretion was very limited, and, in several instances, a chromosome mode close to the tetraploid number was found.

Another potential advantage of the technique described here is that EBV behaves as a polyclonal activator of B cells (26) and can be used to promote the expansion of rare antigen-specific B cells *in vitro* prior to fusion. We also have found that EBV-transformed human B cells are 25-fold more susceptible to hybridization with KR-4 compared with fresh, nontransformed B lymphocytes.

In summary, we have shown that fusion of an Oua^R, $SGua^R$ human B-LCL with EBV-transformed cell lines may be the method of choice for the production of human monoclonal antibody because (*i*) the resulting hybrids are stable ($\geq$7 mo), have a higher cloning efficiency, and produce antibody at a rate at least one order of magnitude higher than EBV-transformed parental cells; (*ii*) there is little restriction as to the type of antibody produced because the antigen-specificity of the molecule is determined initially by the choice of vaccinated human donors; (*iii*) rare antigen-specific B cells in the peripheral blood could conceivably be expanded by EBV transformation prior to fusion, and (*iv*) EBV-transformed lymphocytes fuse preferentially to KR-4 cells compared to nontransformed B cells.

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