Immunoglobulin gene expression in transformed lymphoid cells

(gpt/transformation)

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ABSTRACT   Myeloma, hybridoma, and thymoma cell lines have been successfully transfected for the *Escherichia coli* xanthine-guanine phosphoribosyltransferase gene (*gpt*) by using the plasmid vector pSV2-gpt. The transformed cells synthesize the bacterial enzyme 5-phospho-α-D-ribose-1-diphosphate:xanthine phosphoribosyltransferase (XGPRT; EC 2.4.2.22) and have been maintained in selective medium for over 4 months. Lymphoid cell lines expressing a κ immunoglobulin light chain were obtained by transfecting cells with pSV2-gpt containing a rearranged κ light chain genomic segment from the S107 myeloma cell line. The S107 light chain is synthesized in gpt-transformed J558L myeloma cells and is identical to the light chain synthesized by the S107 myeloma cell line, as judged by immunoprecipitation and two-dimensional gel electrophoresis. Furthermore, this light chain is synthesized and secreted as part of an intact antibody molecule by transformed hybridoma cells that normally secrete an IgG1 (γ,κ) antibody molecule. No light chain synthesis was detected in a similarly transformed rat myeloma or a mouse thymoma line.

Techniques to introduce novel genes into eukaryotic cells provide a powerful tool to study mechanisms of gene regulation and expression. Most studies on eukaryotic gene expression have been conducted in heterologous host cells—i.e., genes have been transfected into cell types (particularly human HeLa and mouse L cells) that normally do not express the gene of interest (1–3). Though a great deal has been learned about eukaryotic regulator sequences with these gene transfer experiments, it would be preferable to transfer genes encoding proteins expressed during differentiation back into the cell type that normally expresses the genes of interest. The appropriate cell type provides protein modification systems, such as glycosyltransferases, necessary to make fully biological functional products. In addition, the appropriate cell type may be used to study tissue-specific regulation of gene expression.

To undertake studies of (*i*) the regulation and expression of immunoglobulin genes, (*ii*) the biosynthesis, chain-assembly, and secretion of immunoglobulin heavy and light chains, and (*iii*) structure–function correlates of antibody molecules, we have explored techniques for transfection of lymphoid cells using the pSV2-gpt vector (4, 5). This DNA can express the *Eco* gpt gene encoding xanthine-guanine phosphoribosyltransferase (XGPRT; 5-phospho-α-D-ribose-1-diphosphate:xanthine phosphoribosyltransferase, EC 2.4.2.22). Cells synthesizing XGPRT can be grown with xanthine as the sole precursor of guanine nucleotide formation (4, 5). Successfully transformed cells can be isolated by their ability to grow in medium containing xanthine and mycophenolic acid, an inhibitor of guanine nucleotide synthesis; if the transformed cell line is hypoxanthine phosphoribosyltransferase-negative (HPRT⁻; IMP pyrophosphate phosphoribosyltransferase, EC 2.4.2.8), transformants can be

selected in hypoxanthine/aminopterin/thymidine (HAT) medium (6). In the present experiments both calcium phosphate precipitation (7, 8) and protoplast fusion (9) techniques have been used to transfect cells.

pSV2-gpt containing a rearranged κ light chain gene (10) was used to transform several cultured lymphoid cell lines. Among the gpt transformants were clones that produce a new immunoglobulin light chain. The light chain produced by these transformed cell lines appears to be identical to the light chain synthesized by the myeloma cell from which the rearranged gene was isolated. Furthermore, in transformed hybridoma cells, this light chain is assembled with an immunoglobulin heavy chain and secreted as a complete antibody molecule.

MATERIALS AND METHODS

Cell Lines. J558L is a spontaneous heavy chain-loss-variant myeloma cell line obtained from the J558 cell line [α,λ; anti-α1-3 dextran (11)] that synthesizes and secretes a λ light chain. Y3-Ag1.2.3 is a HPRT⁻ rat myeloma cell line originally described by Galfre *et al.* (12) that synthesizes and secretes a rat κ light chain. 27-44 is a HPRT⁻ mouse IgG1 anti-dansyl hybridoma cell line (13); and BW5147 is a HPRT⁻, ouabain^r AKR thymoma originally described by Hyman and Stallings (14). Cell lines were maintained in either 10% newborn calf serum in Dulbecco's modified minimal essential medium (DME medium) or 10% fetal calf serum in alpha modified minimal essential medium.

Recombinant DNA Vectors. The plasmid vector pSV2-gpt has been described (4, 5). Fig. 1 shows a partial restriction enzyme map of this vector. A second vector, which is derived from pSV2-gpt, but contains the herpes simplex thymidine kinase promoter inserted 5' of the gpt gene, was constructed by J.-F. Nicolas (unpublished data). pSV2-S107 was constructed by inserting a *Bam*HI fragment containing the entire rearranged phosphocholine-specific κ chain gene from the S107 myeloma cell line (10) into the unique *Bam*HI site in pSV2-gpt. The light chain gene is oriented so that the direction of transcription is opposite to the gpt gene (Fig. 1). The genomic rearranged S107 κ light chain DNA was a gift from M. Scharff.

Transfection by Protoplast Fusion. Protoplasts were prepared essentially as described by Sandri-Goldin *et al.* (9). *Escherichia coli* K-12 strain HB101, containing the appropriate plasmid, was grown at 37°C in Luria broth containing 1% glucose to an absorbance at 600 nm of 0.6–0.8. Chloramphenicol was added to 125 μg/ml, the culture was incubated at 37°C for 12–16 hr to amplify the plasmid copy number, and the cells were harvested by centrifugation. For every 25 ml of culture, 1.25 ml of chilled 20% sucrose/0.05 M Tris·HCl (pH 8) was added;

Abbreviations: XGPRT, xanthine-guanine phosphoribosyltransferase; HPRT, hypoxanthine phosphoribosyltransferase; HAT, hypoxanthine/aminopterin/thymidine; DME medium, Dulbecco's modified minimal essential medium.
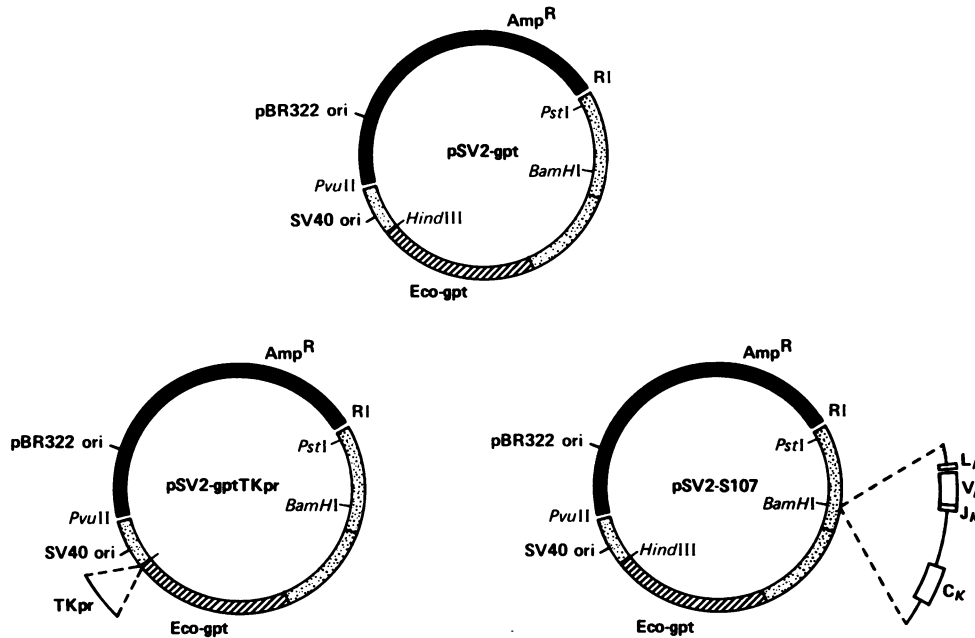
FIG. 1. Structure of the vectors used for lymphoid cell transformations. The diagram of the parental pSV2-gpt plasmid vector was taken from Mulligan and Berg (4, 5): pBR322 DNA is represented by the solid black lines and the plasmid's DNA replication origin and β-lactamase gene are indicated; the *gpt* gene sequence is represented by the hatched segments; simian virus 40 (SV40) sequences are the stippled segments. The SV40 origin of DNA replication (ori) and early promoter are located 5' of the *gpt* sequences. pSV2-gptTKpr has an insertion of 250 base pairs, containing the herpes simplex thymidine kinase promoter, between the *gpt* gene and the SV40 early promoter (unpublished data). pSV2-S107 has a 7-kilobase *Bam*HI fragment, containing the entire genomic S107 light chain gene, inserted into the unique *Bam*HI site of pSV2-gpt. This rearranged light chain gene is oriented in the opposite direction to *gpt* and contains the leader, V, and κ constant region exons as well as flanking 5' and 3' sequences.

the bacteria were suspended and 0.25 ml of lysozyme [a freshly prepared solution of 5 mg/ml in 0.25 M Tris·HCl (pH 8)] was added. After 5 min of incubation on ice, 0.5 ml of 0.25 M EDTA (pH 8) was added and incubation on ice was continued for an additional 5 min. After addition of 0.5 ml of 0.05 M Tris·HCl (pH 8), the bacteria were transferred to a 37°C water bath and were incubated for 10 min. At this time examination of the bacteria with a phase-contrast microscope showed that the vast majority had been converted to protoplasts. The bacteria were diluted with 10 ml of DME medium containing 10% sucrose and 10 mM $MgCl_2$ that was warmed to 37°C. After further incubation for 10 min at room temperature the protoplasts were ready for fusion.

Fusion of protoplasts with suspension cells was effected with a procedure normally used in the production of hybridomas (15). Cell lines were grown to a density of 0.3–1 × 10^6 cells per ml in DME medium supplemented with 10% newborn calf serum. Five milliliters of the protoplast suspension was added to 2 × 10^6 cells in growth medium. The mixture was centrifuged for 5 min at room temperature at approximately 500 × g. The supernate was aspirated and the pellet was resuspended gently in 2 ml of a polyethylene glycol solution [50 g of polyethylene glycol 1,500 (BDH) in 50 ml of DME medium] adjusted to pH 8 with CO_2. After 3 min of centrifugation at 500 × g the polyethylene glycol was diluted with 7 ml of DME medium while resuspending the pellet. After 5 min of centrifugation at 500 × g, the supernate was removed carefully and the cells were resuspended in DME medium containing 10% newborn calf serum and garamycin at 100 μg/ml and were plated either in 96-well or 24-well plates. After 48 hr, cells were diluted with an equal volume of DME medium containing xanthine at 250 μg/ml, hypoxanthine at 15 μg/ml, mycophenolic acid at 6 μg/ml, and 10% newborn calf serum. Every several days, as required, spent medium was aspirated carefully and was replaced with fresh medium containing the same supplements. Colonies of transformants were visible by 10 days. Transformants were maintained in selective medium.

Transfection by Calcium Phosphate Precipitation. Lymphoid cell lines grown in suspension were transfected by calcium phosphate precipitation as described by Chu and Sharp (7). Ten times concentrated HeBS buffer was stored at −20°C

until used, whereupon it was diluted to two times concentrated and adjusted to pH 7.05. Plasmid DNA (80 μg/ml) was made up in 125 mM $CaCl_2$ which was stored as a 2 M stock solution at −20°C. DNA-calcium phosphate precipitates were formed by dropwise addition of the DNA into the HeBS solution. The precipitate formed in 30 min at room temperature. The final DNA concentration was 40 μg/ml.

Cells were washed once in serum-free medium and were suspended directly in the DNA-calcium phosphate precipitate (10^6 cells per 20 μg of DNA per 0.5 ml). This suspension was incubated at 37°C for 30 min and then was diluted 1:10 in serum-containing medium. The cells were plated either into 24-well plates (2 × 10^5 cells per well) or 96-well plates (2 × 10^4 cells per well). Transfection of Y3 cells was done as described by Graham and Van der Eb (8) for adherent cell lines. The DNA-calcium phosphate precipitate was put directly onto the cell monolayer. After 30 min at 37°C, serum-containing medium was added. After 24 hr, half of the medium volume from each culture was removed and was replaced with fresh medium. On days 3, 4, 5, 8, 11, and 14, half the medium volume was removed and HAT medium was added. Transformed colonies were visible between 10 and 21 days.

Immunoprecipitations and Gel Electrophoresis. Immunoprecipitations were done with [^{35}S]methionine-labeled cell lysates and supernates. Biosynthetic labeling procedures have been described (16). Rabbit anti-mouse light chains, rabbit anti-mouse κ light chains, rabbit anti-mouse immunoglobulin, and a hybridoma anti-mouse IgG1 allotype antibody were used for immunoprecipitations. *Staphylococcus aureus*, Cowan strain 1 (IgGsorb; Enzyme Center, Boston) was used to coprecipitate the antigen–antibody complexes (16).

One-dimensional $NaDodSO_4$/polyacrylamide slab electrophoresis and two-dimensional nonequilibrium gradient gel electrophoresis were done as described (17). Autoradiography of polyacrylamide gels was with preflashed XAR-5 film and fluorography by using sodium salicylate (18).

RESULTS

Transfection Frequencies. The frequency at which stable transformed lymphoid cell lines were generated was influenced

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by every parameter tested. Different cell lines and different vectors produced different transformation frequencies. Moreover, the two DNA delivery procedures, protoplast fusion and calcium phosphate precipitation, yielded different transformation frequencies. Tables 1 and 2 summarize the results by using protoplast fusion and calcium phosphate precipitation, respectively.

Under the present experimental conditions, BW5147 appears to be the least competent recipient of the cell lines tested, having a transformation frequency of approximately 10^{-6}. Y3 and 27-44 yielded frequencies in the range of 0.3 to >5 × 10^{-6}. In the present experiments, J558L yielded the highest frequency with the range of 3 × 10^{-6} to >10^{-4}. Protoplast fusion appears on balance to be a more efficient delivery system than calcium phosphate precipitation.

A striking feature of these results is the enhanced transformation frequency for gpt obtained with the light chain-containing vector, pSV2-S107. This dramatic increase is evident when the pSV2-S107 vector was used with the J558L and Y3 myeloma cell lines; transformation with this recombinant was 5- to at least 10-fold greater than that obtained with the other vectors. Transformation of the hybridoma 27-44 cell line was increased only about 2-fold with pSV2-S107. The sequence(s) in the pSV2-S107 insert that is responsible for the enhanced transformation frequency must yet be mapped. Transformation of the Y3 cell line was occasionally greater with pSV2-gptTKpr than with pSV2-gpt (Table 2). Regardless of which vector was used, BW5147 transformants were detected only at very low frequencies. The amount of XGPRT activity in cell lines stably transformed by the three recombinant plasmids was not significantly different (Fig. 2 and data not shown).

XGPRT Activity. The transformed cell lines expressed the Eco gpt gene, as measured by the presence of XGPRT activity in the cell lysates. E. coli XGPRT can be distinguished from mammalian HPRT activity by its different electrophoretic mobility (4, 5). In cells selected for resistance to mycophenolic acid, both the cellular HPRT and bacterial XGPRT activities were detectable (Fig. 2). Cells lacking their own HPRT activity and selected for gpt in HAT medium had only the bacterial enzyme activity (Fig. 2).

Immunoglobulin Light Chain Expression. The organization of exons in the S107 genomic light chain gene is shown in Fig. 1. To produce the S107 light chain protein from this gene, two introns must be processed from the primary mRNA transcripts and the leader polypeptide removed by post-translational cleavage. For secretion of the light chain as part of an intact antibody molecule, the newly synthesized light chain must fold and assemble with an immunoglobulin heavy chain to form an H_2L_2 tetramer. This also involves the formation of interchain disulfide bonds.

Table 1. Transformation of lymphoid cell lines with the pSV2-gpt vectors by using protoplast fusion

Vector	Cell line			
	J558L		BW5147	
pSV2-gpt	21/288*	27/36†	0/76*	1/48†
pSV2-gptTKpr	10/190*	24/36†	0/80*	1/48†
pSV2-S107	186/192*	36/36†	4/96*	8/48†
	149/192‡			

Results are from three experiments and are expressed as the number of culture wells having stable transformants.
* After protoplast fusion cells were plated in 96-well culture dishes at 10^4 cells per well.
† Cells were plated at 10^5 cells per 2.0 ml of culture in 24-well dishes.
‡ Cells were plated at 5 × 10^3 cells per well in 96-well culture dishes.

Table 2. Transformation of lymphoid cell lines with the pSV2-gpt vectors by using calcium phosphate precipitation

Vector	Cell line		
	Y3	27-44	BW5147
pSV2-gpt	3/48*	10/192†	1/192†
pSV2-gptTKpr	19/48*	10/192†	1/192†
pSV2-S107	47/48*	43/288†	0/192†

Results are from three experiments with the Y3 cell line and two experiments with 27-44 and BW5147 cell lines.
* Cells were plated in 24-well culture dishes at 2 × 10^5 cells per well.
† Cells were plated in 96-well culture dishes at 4 × 10^4 cells per well.

Of the four cell lines stably transformed with pSV2-S107, the J558L cell line synthesized, but did not secrete, the S107 κ light chain. However, this cell line was not expected to secrete the newly made light chains because heavy chain-loss-variants of the S107 myeloma cell line also do not secrete endogenous light chains (M. Scharff, personal communication). Transformants of the 27-44 hybridoma cell line synthesized and secreted the S107 light chain. Moreover, the S107 light chain was assembled into tetrameric H_2L_2 immunoglobulin molecules with the endogenous γ1 heavy chain and was secreted. Twelve independently transformed Y3 and seven BW5147 cell lines did not produce detectable amounts of the S107 light chain, as judged by immunoprecipitation and gel analyses. XGPRT analyses verified that these cells were, indeed, transformants.

Autoradiograms of two-dimensional polyacrylamide gels showing the apparent M_r and charge of the light chains produced by J558L and 27-44 transformants are shown in Figs. 3 and 4. The two-dimensional gel pattern of the S107 light chain synthesized by S107 myeloma cells is included to show that the transformed cell lines produced a light chain that is identical in apparent M_r and charge. The two-dimensional gel patterns also show that the leader polypeptide was removed in transformed cell lines that expressed the light chain. This indicates that proper transcription, mRNA, and protein processing occur in the transformants. Transcription of the S107 light chain gene probably occurs from its own promoter, because the light chain gene is oriented opposite to the direction of the SV40 early promoter (see Fig. 1).

The antibodies secreted by 27-44 transformants were immunoprecipitated with both hybridoma anti-IgG1 allotypic antibody and rabbit anti-mouse light chain antisera. Both reagents precipitated the S107 light chain (data not shown). Sequential precipitation, first with the hybridoma anti-IgG1 antibody and
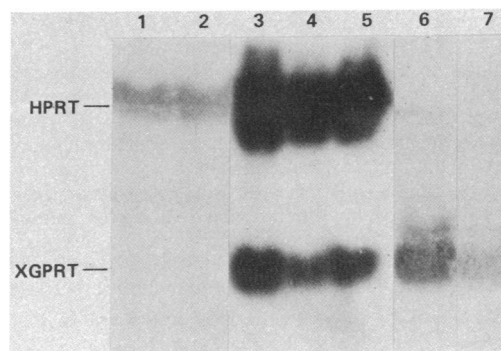

FIG. 2. XGPRT and HPRT production in transformed lymphoid cell lines. Enzyme analyses were done as described by Mulligan and Berg (4, 5). Lanes: 1 and 2, electrophoretic mobility of mammalian HPRT; 3–5, J558L cell transformants; and 6 and 7, transformants of the 27-44 cell line. Because 27-44 is a HPRT⁻ cell line, only XGPRT is present.
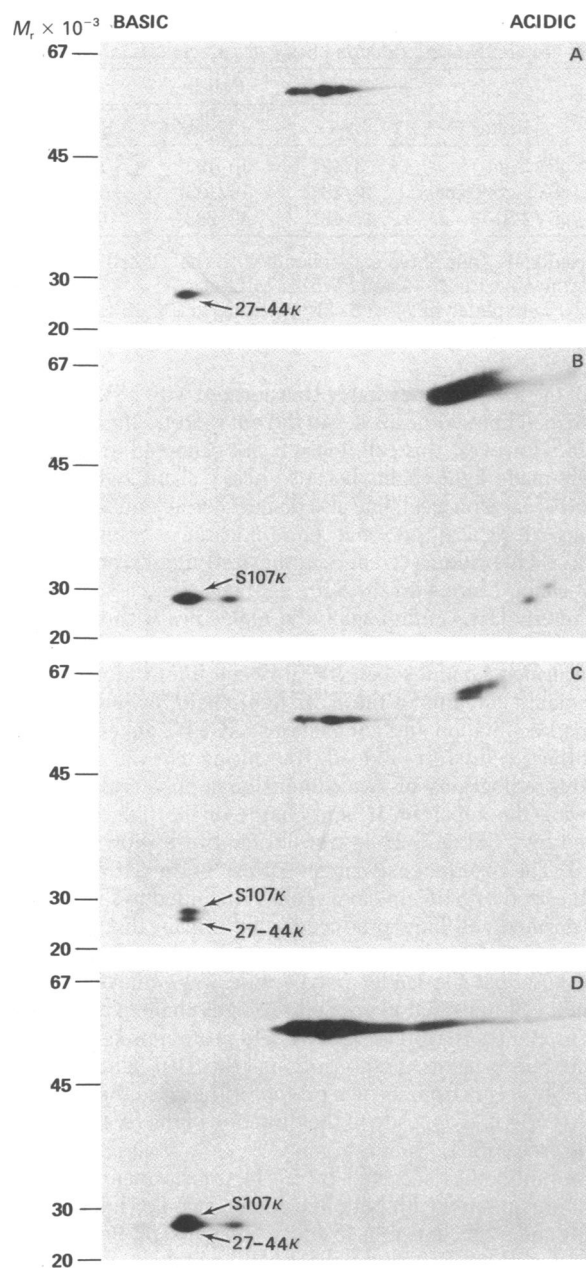
FIG. 3. S107 light chain produced by transformed 27-44 cells. Autoradiograms of two-dimensional gels of the light and heavy chains produced by parental and transformed cell lines are shown. (A) Parental 27-44 IgG1 anti-dansyl antibody immunoprecipitated with an anti-IgG1-specific hybridoma antibody. Both the γ1 heavy and κ light chains can be seen. (B) S107 IgA antibody immunoprecipitated with a rabbit anti-IgA antiserum. The α heavy chain was distinguished clearly by charge and apparent M_r from the γ1 heavy chain in A. (C) A mixture of the immunoprecipitates of A and B. The two κ light chains can be seen as distinct spots (indicated by arrows) having nearly identical charge but different apparent M_r. (D) Immunoprecipitate of a transformed 27-44 cell line. Only the γ1 heavy chain was present, but two light chains can be seen. In this case the amount of S107 light chain was considerably lower than in the artificial mixture shown in C.

then with the rabbit anti-κ antisera, indicated that little, if any, free S107 light chain was secreted by these cells. This shows that the S107 light chain is assembled with the γ1 heavy chain into an intact antibody molecule.

Different amounts of S107 light chains were produced when a number of independent J558L and 27-44 transformants were
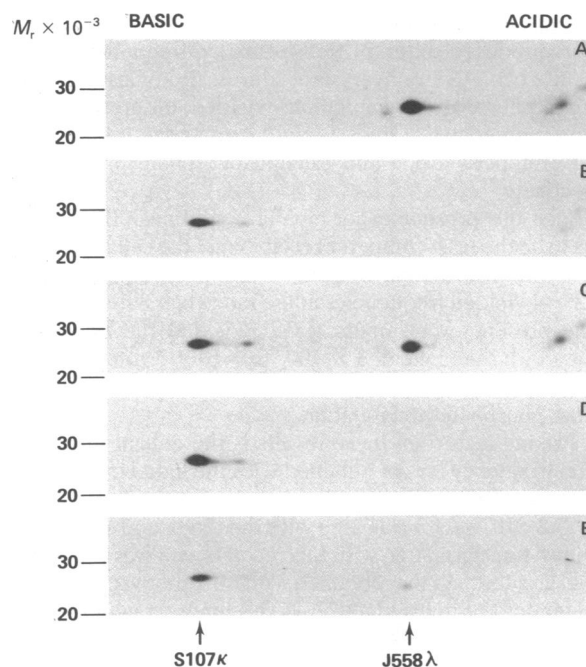

FIG. 4. S107 light chain produced by transformed J558L cells. Autoradiograms of two-dimensional gels of light chains immunoprecipitated from cell lysates of J558L transformed with pSV2-S107 DNA are shown. Because J558L does not produce a heavy chain, only the light chain portions of the two-dimensional gels are shown. (A) λ light chain produced by the parental J558L cell line. (B) S107 κ light chain. (C) A mixture of the two light chains. The κ and λ light chains are distinguished on the basis of both charge and apparent M_r. (D and E) Two independently derived J558L cell lines transformed with pSV2-S107 DNA. The transformant examined in D only appears to produce larger quantities of the S107 κ light chain than the endogenous J558 λ light chain, because the S107 κ chain is synthesized and remains in the cytoplasm, while the J558 λ chain is synthesized and secreted.

compared. Amounts varied from barely detectable to quantities equal to endogenous light chain. This variation may be due to the chromosomal region where the light chain has integrated. It also could result from different copy number of the light chain gene in different transformants. Quite possibly, mutations or deletions of sequences needed for the expression of this gene could have occurred during transformation or subsequent to integration of the light chain sequence. Further studies are needed to determine the cause of this variation and why light chain expression does not occur in Y3 or BW5147 cell lines transformed with the same light chain gene vector.

DISCUSSION

These experiments show that it is possible to use two methods, calcium phosphate precipitation and protoplast fusion, to introduce genes into lymphoid cells. With pSV2-gpt containing the gene for an immunoglobulin light chain (pSV2-S107) both methods give rise to transformants that synthesize bacterial XGPRT and the murine light chain. Higher transformation frequencies are seen following protoplast fusion. Indeed, by using protoplast fusion and the pSV2-S107 plasmid, transformants can be obtained at a frequency of greater than 10^{-4}. Transformation frequencies are lower when using the other plasmids or calcium phosphate precipitation. Because mycophenolic acid resistance or reversion of the HPRT⁻ phenotype do not occur spontaneously in the cell lines used, stable transformation, at even low frequencies, can be detected.

A surprising result is the increased frequency of *gpt* transformation when the S107 light chain is incorporated into the

pSV2-gpt vector. This enhancing effect occurs with both the rat and mouse myelomas. A similar increased transformation frequency has been observed with a bovine papillomavirus vector containing the human β-globin region sequences (19). At present, the mechanism for the increased transformation frequency in both cases is obscure. Possibly, the chromosomal DNA provides an origin of DNA replication, which permits the plasmid to replicate within the transformed cell and increases the transformation frequency. Transcription from the immunoglobulin promoter cannot be essential for the increased transformation frequencies because deletion of the fragments that are presumed to contain the immunoglobulin promoter region does not abolish the enhancement of transformation. It also is possible that pSV2-S107 is more efficient for transformation because of increased XGPRT production; this seems unlikely because there are no consistent differences in enzyme levels in the stable transformants obtained with either vector.

DNA-mediated gene transfer into lymphoid cells may permit a study of the regulation and expression of immunoglobulin genes in cells in which they normally are synthesized. It may be possible to examine the basis for differential immunoglobulin gene expression at different stages of lymphocyte differentiation. Cell lines in which immunoglobulin synthesis ca_n be induced (20–22) are suitable hosts to determine if the transduced immunoglobulin genes also are responsive to those signals. Studies with cells transformed with genetic elements that are inducible by steroid hormones demonstrate that transduced DNA can respond, if the cell contains the appropriate receptors (23).

A question of central importance is what determines the utilization of various promoters and thus the synthesis of defined proteins in certain cell lines. In our experiments light chains are efficiently produced in both transformed mouse myeloma and hybridoma cell lines. However, light chain production did not occur in either a rat myeloma or a mouse thymoma. The inability of the immunoglobulin promoter to function in a different species has been reported by Falkner and Zachau (24). The lack of production of mouse immunoglobulin in a rat myeloma is surprising because mouse myelomas have been used to fuse to rat myelomas to produce hybrid cells that synthesize both rat and mouse immunoglobulin molecules (25). The possibility that the S107 light chain is synthesized but rapidly degraded in the Y3 myeloma has not been excluded.

There is evidence that differentiated cell types express immunoglobulin genes to varying levels. For example, somatic cell hybridization of myelomas yields hybridomas that produce antibodies, whereas thymomas yield hybrid cells with T-cell phenotypes (26). Furthermore, hybridization of myelomas with non-B cells results in cessation of immunoglobulin production (26, 27). The lack of light chain expression in the transformed thymoma may reflect tissue-specific gene regulation. It is important to determine if immunoglobulin gene expression in the nonexpressing mouse thymoma and rat myeloma cell lines is regulated at the level of transcription, RNA processing, translation, or rapid protein turnover.

The study of the structure and function of the immunoglobulin molecule has been of great interest, both because of the ability of immunoglobulin to react with a diverse family of ligands and also because of the biologic importance of antibody molecules. Initially, the study of immunoglobulins was limited to the study of heterogeneous serum pools after immunization. The advent of myelomas, and more recently hybridomas, has permitted the study of homogeneous populations of antibodies. DNA-mediated transfection and immunoglobulin gene expression is an important tool to permit the study of immunoglobulin

molecules. By using this technique, it should be possible to study the function of both novel chain combinations and novel chain structures. In vitro site-specific mutagenesis techniques can be used to construct specific mutations in immunoglobulin genes that can be expressed after transfection. Because significant quantities of immunoglobulin are produced in the transformants, sufficient quantities of protein necessary for detailed analyses should be obtained.

Note Added in Proof. After this paper was submitted for publication, we learned that Douglas Rice and David Baltimore have reported similar results with a different κ light chain gene and different lymphoid cell recipients (28).

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