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Cell-surface antigens expressed on L-cells transfected with whole DNA from non-expressing and expressing cells

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We have shown previously[1] that transfection of mouse L-cells with DNA from JM, a human T-cell line expressing certain T-cell differentiation antigens, yields stable transfectants expressing one or another of these antigens. The identities of the antigens were confirmed by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. We now report that our procedure—co-transfection[2] with the chicken thymidine kinase gene (*tk*) and whole cellular DNA, selection with hypoxanthine–aminopterin–thymidine (HAT)[3], and staining of the cells with fluorochrome-conjugated monoclonal antibodies and fluorescence-activated cell-sorter (FACS) selection—yields transfectants expressing a variety of cell-surface molecules (19 of 21 investigated), most at a frequency of about one per 10^3 Tk$^+$ transformants. Of these, 9 of 12 were transferred and expressed as readily using DNA from cells which did not express the cell-surface antigens as from tissues or cells that did express them.

We performed Tk co-transfections with DNA from human placenta, kidney, a renal cell carcinoma derived from the same individual as the kidney, and LCL-721, a human B-lymphoblastoid cell line. None of these tissues expresses serologically detectable Leu-1 or Leu-2, except LCL-721, which expresses Leu-1; in none is Leu-2 messenger RNA detectable by Northern blotting using cloned Leu-2 complementary DNA as probe[28]. DNA from JM, which expresses these genes, served as a positive control.

Tk$^+$ transformants were selected by FACS for expression of Leu-1 or Leu-2. As positive controls, we selected transfectants expressing HLA class I antigens or human transferrin receptor, as these are constitutively expressed in all of the tissues we studied. We recovered Leu-2$^+$ transfectants from all of the human DNA sources, at a frequency of about one per 10^3 Tk$^+$ transformants (Table 1). We also recovered, though less frequently, transfectants expressing either Leu-1 (from both antigen-expressing and non-expressing sources) or human transferrin receptor.

In our original work with JM DNA, one of the Leu-2$^+$ transfectants expressed very high, but unstable, levels of surface Leu-2. The gene for Leu-2 was highly amplified in this transformant[4,28]. We have now found that 11 of 20 independent Leu-2$^+$ transfectants exhibit similarly high and heterogeneous levels of Leu-2. In 10 of these cases, further rounds of selection for the most brightly staining cells have yielded at least a 4-fold increase in antigen expression, resulting in cells that are now over 20-fold brighter than 'normal', stable Leu-2$^+$ transfectants. In some cases, we have confirmed Leu-2 gene amplification by quantitative filter hybridization of RNA[5] and DNA[6] to a cloned Leu-2 gene probe[28].

To further our study using multiple organ and tissue sources and to determine how many of the antigens for which we had available antibodies could be transferred and expressed, we extended our work to mouse DNA transfections. We studied 11 murine cell-surface antigens. Nine are believed to be monomeric glycoproteins. One, Lyt-2, occurs primarily as a heterodimer[7]. The two chains may be encoded by one gene or

Table 2 Murine lymphoid antigens detected on transfected L-cells using monoclonal antibodies

Antigen	Tissue source of DNA Spleen	Kidney	Muscle	L(Tk$^-$)
Ly-1	3/3*	2/2	2/2	5/6
Ly-2	2/3	2/2	2/2	4/6
BLA-2	2/3	2/2	2/2	3/6
ThB	3/3	2/2	2/2	ND
FcR(ref. 26)	2/3	0/2	1/2	ND
Thy-1	1/3	2/2	1/2	ND
L3T4a(ref. 27)	1/3	ND	1/2	4/6
H-2^d	3/3	2/2	ND	ND

* No. of dishes yielding positive transfectants/total no. of dishes analysed.

Table 1 Human antigens detected on transfected mouse L-cells using monoclonal antibodies

Antigen	DNA donor: Placenta	Kidney	Renal cell carcinoma	LCL-721 (B-lymphoblastoid)	JM (T-cell lymphoma)
HLA (W6/32)	ND	3/6†	6/6	4/4	2/2
Leu 2	6/6	3/6	1/6	7/8	3/6
(Leu 2)*	(4/6)	(1/3)	(1/1)	(3/7)	(2/3)

Isolation of transfectants expressing novel cell-surface antigens: L(Tk$^-$) cells[22] were grown in 100-mm tissue culture dishes (Lux) in Dulbecco's modified Eagle's medium with 10% fetal calf serum. DNA was prepared from various tissues as described previously[23]. Briefly, the tissues were pulverized under liquid nitrogen, dissolved in 1% sarkosyl, 50 mM EDTA, 0.2 mg ml^{-1} proteinase K (Merck), followed by treatment with RNase, extraction with phenol followed by chloroform, and caesium chloride equilibrium gradient centrifugation. The purified DNA was recovered (and sterilized) by ethanol precipitation and dissolved in 10 mM Tris-HCl, 1 mM EDTA. Transfections were carried out using a modification of the protocol of Wigler *et al.*[24]. In most cases the DNA was sheared before precipitation by one passage through a 23-gauge needle. In all cases, 1 μg of Tk-bearing plasmid (the chicken thymidine kinase gene, cloned into the plasmid pBR322 and propagated in the host *Escherichia coli* GM48 (*dam$^-$mec$^-$*)[25] was used with 20 μg of genomic ('carrier') DNA per dish of 10^6 cells. Transfectants were selected for expression of Tk genes by growth in HAT[3]. They were then selected for expression of specific antigens by staining with fluoresceinated monoclonal antibodies and FACS sorting, as described elsewhere[1]. Initial dishes of transfectants were pooled, if necessary, to form dishes of 1,000–1,500 Tk$^+$ clones each. These were then collected, and each dish was divided into aliquots of 10^6 cells, one aliquot for each reagent to be screened. Staining was done at nominally saturating concentrations of antibody, in sterile 96-well V-bottom culture trays. In the initial round of sorting, cells falling within arbitrary 'gates' representing the positively staining cells were sorted into pots and then regrown for further analysis. In some cases it proved possible to clone positively staining cells directly from the initial culture. In most cases it was necessary to enrich with one or more rounds of 'pot-sorting' for bright cells before it became feasible to clone true positives into individual wells.

W6/32 is an anti-HLA framework monoclonal antibody that is specific for HLA heavy chains even when they are in association with mouse β_2-microglobulin[20,21]. ND, not determined.

* Amplified phenotype (no. amplified/no. positive).

† No. of dishes yielding positive transfectants/total no. of dishes analysed.

Table 3 Expression of mouse genes in transfected L-cells requires mouse DNA

DNA source	Human Leu-2a	Human NGF receptor	Antigen Rat NGF receptor	Mouse 30-E2	Mouse L3T4a
PC12(rat)	ND	ND	5/8*	0/8	0/8
A875(human)	4/6	1/6	ND	0/6	0/6
Peripheral blood lymphocytes (human)	6/6	ND	ND	0/6	0/6

PC12 is a rat chromocytoma expressing NGF receptor; A875 is a human melanoma expressing NGF receptor but not Leu-2. Transfectant cultures were subjected to at least four rounds of sorting and regrowth before being typed as negative. In previous experiments with 30-E2 and L3T4a, no more than two rounds of FACS selection had ever been required to obtain cultures that were > 90% enriched for positive cells, when there were positives present in the starting dishes of transfectants. We note that the results with A875 provide another example of efficient transfer of Leu-2 using DNA from a non-expressing source.

* No. of dishes yielding positive transfectants/total no. of dishes analysed.

by closely linked genes. In the case of H-2, the host cell[8] or the culture medium serum[9] can supply β_2-microglobulin to complement the heavy chains encoded by the donor DNA, and permit surface expression.

We used DNA from BALB/c mice, as they differ at several serologically detectable loci from the mouse strain (C3H) from which L-cells were derived[10]. Because all of the antigens tested are present on at least 10% of spleen cells[11,12], we used spleen DNA as our positive standard. None of these antigens is known to be expressed on any of the other tissues used. The various DNA sources differed little in yield of transfectants for 8 of the 11 antigens tested (Table 2). For the other three antigens (T200, BLA-1 and 30-Fl, not tabulated), we recovered only one transfectant, preventing meaningful comparisons.

For heuristic purposes, we also used DNA from the L(Tk$^-$) line itself. We used only those antibodies with which we had previously most readily detected transfectants. We recovered L-cell transfectants positive for the target antigens as frequently with L-cell DNA as with the other DNAs (Table 2).

It is conceivable that the murine antigens are produced by L-cell genes that are active in rare variants in normal L-cell cultures, or which are activated by the transfection process. We therefore transfected L-cells with human or rat DNA and looked for cells expressing murine antigens. We did not isolate any transfectants expressing the murine antigens tested, although we did obtain transfectants expressing Leu-2 or rat nerve growth factor (NGF) receptor at expected frequencies (Table 3). Furthermore, in the case of Ly-1, Lyt-2 and H-2 antigens, the BALB/c DNA-derived transfectants stain with antibodies specific for the donor allotype, showing directly that they express transferred, and not endogenous, genes.

Two antigens for which we obtained transfectants were Lyt-2 (see Table 2) and 30-Fl (data not shown). In the normal animal, each is associated with another cell-surface determinant (Lyt-2 with Lyt-3 (ref. 11) and 30-Fl with BLA-1 (R. Hardy, unpublished)). None of the Lyt-2$^+$ transfectants stain with anti-Lyt-3, nor do they contain the Lyt-3 polypeptide when examined by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. However, the one 30-Fl$^+$ transfectant isolated also expresses BLA-1. As co-transfer of two unlinked genes is very unlikely[13], and in light of their association on normal cells, we propose that 30-Fl and BLA-1 are encoded by one gene or by two very tightly linked genes.

The BLA-2$^+$ transfectants expressed extremely high levels of antigen, enabling us to perform an initial characterization of the molecule. We immunoprecipitated a 94,000 molecular weight protein consisting of one polypeptide chain. This molecule has now been identified on various murine B-cell lines known from staining studies to be BLA-2$^+$ (R. Hardy and G. Calicchio, unpublished).

We believe this to be the first demonstration that several genes can be stably transferred in active form using DNA from tissue sources not expressing those genes. Further, in the case of most of the genes we analysed, DNA from non-expressing sources appears to be as efficient in transfection as DNA from expressing tissues.

Other studies[14-16], using cloned genes, show an inverse correlation between the activity of genes in gene transfer and their degree of cytosine base methylation. The means by which the genes in our study were originally turned off may not depend on this type of modification, or they may be insufficiently modified to keep them turned off in L-cells. We have readily recovered Leu-2$^+$ transfectants using the highly methylated[17] DNA from human sperm. However, we have not recovered any Leu-2$^+$ transfectants using DNA from certain human choriocarcinoma cell lines (S. Alberti, C.H. and L.A.H., in preparation); these are also the only DNA sources we have used which do not express antigens of the major histocompatibility complex. We are now investigating whether there is a special mechanism for turning off genes in these cells.

Enhancer sequences have been shown to be important in the expression of certain cloned genes in transfection systems[18,19]. These *cis*-acting elements increase transcription rate and accuracy, and seem to act in a highly tissue-specific manner. The stringent tissue-specificity attributed to the expression of certain differentially expressed genes (for example, immunoglobulins) in gene-transfer systems does not seem to hold in L-cells for the genes we have transferred using total cellular DNA.

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