Amplification of a gene coding for human T-cell differentiation antigen

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Using previously isolated mouse L-cell transferrants for the human T-cell differentiation antigen Leu-2, we now report the first example of spontaneous gene amplification for membrane antigens. The Leu-2 (or T8) antigen is normally expressed on T lymphocytes and has strong suppressor functions. Cells of a Leu-2 transfected clone were stained with fluorescently-tagged monoclonal anti-Leu-2, and the brightest 0.1-0.3% of cells were visibly separated using a fluorescence activated cell sorter (FACS) and have typical suppressor functions. Cycles of growth of these selected transferrants were repeated six times, resulting in a population of cells that, compared with the starting population, stains 40 times higher for Leu-2 and whose DNA transforms 20 times more efficiently for Leu-2. In addition, these cells have 10- to 50-fold amplified human DNA sequences and numerous double minute chromosome fragments, a common indicator of gene amplification in mouse cells.

Following co-transfection of mouse L cells (TK-) with the herpes simplex thymidine kinase (TK) gene and total human DNA from JM, a human T lymphoma line, we selected Leu-2 and other human lymphocyte membrane antigen transferrants by FACS sorting1. We found the frequency of transferrants for Leu-2 to be about 10^{-30} of the TK- cells selected in hypoxyanthine-aminopurine-thymidine (HAT) medium. Although most cloned transferrants had narrow ranges of antigen density per cell, one of the first four Leu-2 transferrants found was strikingly more variable in the amount of Leu-2 antigen per cell. Further, this transferrant, J10, had a mean Leu-2 staining per cell that was seven times greater than that of the other transferrants.

To investigate whether the increased Leu-2 expression on the transfected cells was due to gene amplification, we sorted the brightest 0.3% of the cells aseptically with the FACS. These cells were grown, reanalysed, and the brightest cells again sorted and regrown. Each round took about 3 weeks. After six rounds of selection the mean fluorescence of the cells was about 40 times greater than that of the original cloned transferrant J10 (Fig. 1); the mean brightness approximately doubled with each sort. Curiously, we found no further increase in mean fluorescence after 4 cycles of sorting. Levels of sorting were not determined.

The presence of double minute (DM) chromosomes is characterized of mouse cells that have amplified sequences4. DMs are self-replicating acentric chromosomal fragments that are distributed unequally in daughter cells at mitosis. We examined metaphase spreads of J10-6 cells (the J10 transferrant after six cycles of sorting) and could clearly see DMs in most spreads (Fig. 2). Variation from <10 to >100 in the number of DMs per spread was noted, which is like-other counts of DMs in amplified mouse cell lines4.

Because a Leu-2 gene probe does not yet exist for quantitation of Leu-2 gene copies, we used a biological assay, quantitative transfection, to test whether the Leu-2 gene number had increased. We found the transfection frequency of Leu-2 into mouse L cells to be roughly 15-20 times higher per μg of DNA using DNA from the amplified line J10-6, than the frequency using JM or J10 DNA (Table 1). We next titrated J10-6 DNA in a transfection experiment with different amounts of carrier DNA. When 1 μg of J10-6 DNA was used with 20 μg carrier (L cell) DNA per dish, Leu-2 transferrants could be detected on every dish (four dishes per experiment). No Leu-2 transferrants were detected with 1 μg of JM DNA with the same amount of carrier DNA. This indicated that the Leu-2 gene was amplified at least 20-fold.

Analyses of DNA in stable transferrants after introduction of specific genes into cells have shown that physically unlinked DNA segments tend to be physically linked in the transferrants5. We would therefore expect that Leu-2 DNA, other human DNA sequences and TK sequences would be ligated together in transferrants generated by co-transfection of mouse L cells with human DNA and the TK gene. We found indeed that all human DNA and many of all TK sequences were amplified in the J10-6 cells. Southern blots of DNA from J10, L cells and J10-6 were probed with human repetitive DNA (Fig. 3) or with the TK gene. Bands of the same size that were detected by human repetitive DNA probes in J10 were also present in J10-6 but with greatly increased intensity. We titred the amount of J10-6 DNA on the blot to see how much J10-6 DNA was required for the band intensities to be equal to those obtained with 10 μg of J10 DNA and found that for most bands 0.2-0.3 μg of J10-6 DNA was roughly equivalent to 10 μg of J10 DNA; thus there was about a 30- to 50-fold increase in copy.
expression on the cell surface. The sum of the sizes of the visible human repetitive probes, selection gives us an estimate of the amplified unit of at least 200 kilobase pairs.

Several pieces of evidence lead us to conclude that the structural gene for Leu-2 is amplified in the selected cells expressing high levels of Leu-2: (1) the transfection frequency for Leu-2 using J10-6 DNA is 15-20 times greater than with the same amount of donor JM lymphoma DNA or J10 DNA, (2) double minute chromosomes, known to occur with gene amplification in mouse cells, are present in J10-6 cells, (3) the introduced human or TK DNA integrated in the L-cell genome is amplified 10-50 times based on Southern blots with human repetitive DNA or TK as probes. Spontaneous amplification for the Leu-2 gene in a mouse L-cell transferrant may be a fairly common event since at least 4 of 20 independent, FACS isolated, Leu-2 transformed clones showed an increased and unstable pattern of antigen expression. For each of the four cultures we were able to select cells with greater antigen expression by FACS selection of the brightest stained cells. Preliminary work indicates that growing cells in HAT medium versus non-HAT medium is more effective for this process.

FACS has been used for selecting cells with other kinds of amplified sequences. Johnston et al. selected for amplification of dihydrofolate reductase (DHFR) in CHO cells by multiple cycles of sorting the brightest cells after staining with fluorescent methotrexate, an inhibitor of DHFR. This group obtained cells that had spontaneously amplified the DHFR gene 50-fold. With suitable amplified probes, selection for amplification of other genes may be made in a similar manner.

Cells with amplified copies of a gene coding for a cell-surface antigen could be very useful for cloning this gene. Cloning by cDNA methods could be simplified because the levels of mRNA are almost certainly increased in amplified cells. For example, Caskey et al. isolated a cDNA clone corresponding to the HPRT gene using a neuroblastoma line with 40-fold amplification. Double minutes could be isolated and used for the production of genomic libraries enriched for amplified sequences. Cells with increased amounts of antigen expression should also make protein purification for structural and functional studies of cell-surface antigens easier.

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Table 1 Transformation frequency of Leu-2 into mouse L cells

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<tr>
<th>DNA donor cell</th>
<th>No. Leu-2 Cells* per 10^4 TK&quot; cells per dish</th>
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<tr>
<td>JM (T-cell lymphoma)</td>
<td>6, 17, 3, 6</td>
</tr>
<tr>
<td>J10 (Leu-2 transformant)</td>
<td>9, 10</td>
</tr>
<tr>
<td>J10-6 (J10 cells after six cycles of sorting)</td>
<td>97, 120, 157</td>
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Each dish was seeded with 10^6 cells and overlaid with 20 μg total DNA and 1 μg of pBR322 plasmid containing the TK gene. Selection of transferrants was as described previously.1

* Number of cells with fluorescence intensity above background. Background was fluorescence intensity of cells stained with second step antibody alone.

number of the Au marked human sequences detected in J10-6. A few of the higher molecular weight bands, however, were amplified only about 10-fold. Southern blots with TK as a probe showed a similar 30- to 50-fold amplification (data not shown). Thus DNA sequences transfected into the L cells are amplified to approximately the same extent as the increase in Leu-2