RAPID COMMUNICATION

Metabolic Correction of Congenital Erythropoietic Porphyria by Retrovirus-Mediated Gene Transfer Into Epstein-Barr Virus-Transformed B-Cell Lines

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Congenital erythropoietic porphyria (CEP) is an inherited metabolic disorder resulting from the accumulation of porphyrins because of defective uroporphyrinogen III synthase (UROIIIS). This autosomal recessive disorder is phenotypically heterogeneous with respect to the age of onset and the severity of the symptoms. Different exonic point mutations in the UROIIIS gene have been identified, providing phenotype-genotype correlations in this disease. Severe cases may be treated by bone marrow transplantation and are potential candidates for somatic gene therapy. Epstein-Barr virus-transformed B-cell lines from patients with CEP provide a model system for the disease. We have used retrovirus-mediated expression of UROIIIS to restore enzymatic activity in a B-cell line from a patient. We have also demonstrated the metabolic correction of the disease, i.e., porphyrin accumulation into the deficient transduced cells was reduced to the normal level. These data show the potential of gene therapy for this disease.

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of selection in 0.4 mg/mL G418 for 2 weeks and then maintained in medium containing 1 mg/mL G418 for 10 weeks.

For retroviral supernatant infection, 4 mL of the filtered supernatant obtained from producer cells was added to the 2 × 10⁶ target cells in the presence of 8 µg/mL polybrene during 3 hours for 6 consecutive days (2:1 colony-forming units/cell ratio). Selection was performed as described above.

Southern blot analysis. High molecular weight DNA was extracted from lymphoblastoid cells by the usual methods and analyzed by gel blot hybridization. Ten micrograms of genomic DNA was digested with SacI; this enzyme cuts in both LTR regions in our construct and produces a 3.6-kb band from the LUSN vector (Fig 2). The resulting fragments were separated on a 1% agarose gel and transferred onto nylon membrane (Hybond N'; Amersham, Les Ulis, France). Known amounts of digested plasmid DNA (20, 10, and 5 pg) mixed with control LB DNA (10 µg) were run on the same gel. Filters were hybridized with a 780-bp human UROIIIS cDNA fragment that was labeled with [32P]dCTP by random priming (Multiprime DNA labeling system; Amersham). The vector copy number per genome was estimated by comparison of the amount of radioactivity obtained with this enzyme cuts in both LTR regions in our construct with the amount of radioactivity (Phosphor Imager; Molecular Dynamics, Sunnyvale, CA). The radioactivity obtained with 10 pg of plasmid DNA corresponds to the presence of one copy of the human genome when 10 µg of genomic DNA is used for the blot.

Determination of UROIIIS enzyme activity. A total of 10⁴ to 10⁶ LB cells (depending on the specific enzymatic activity) were microfuged for 30 seconds and the pellets were washed with phosphate-buffered saline (PBS). The cells were resuspended in 250 µL of UROIIIS lysis buffer (100 mmol/L Tris-Cl, pH 8.0, containing 1 mmol/L EDTA and 1% Tween-20), disrupted by sonication on ice, and microfuged for 5 minutes. Supernatants were assayed for UROIIIS activity as described elsewhere. One unit of UROIIIS activity was defined as the amount of enzyme that formed 1 nanomole of uroporphyrinogen III per hour at 37°C.

Porphyrin accumulation in lymphoblastoid cells and in the medium. Experiments were performed in polystyrene multiwell trays seeded with 5 × 10⁵ cells/well. LB were exposed to 0.3 mmol/L and 0.6 mmol/L δ-aminolevulinic acid (ALA; Sigma) for 24 and 48 hours. The medium was then kept, the cells were washed with PBS, and the porphyrins were extracted from the cells and the culture medium with 1 mol/L HClO₄/CH₃OH (1:1, vol/vol). The total porphyrin concentrations of the extracts were determined by spectrofluorimetry (excitation, 404 nm; emission, 595 nm) using an uroporphyrin I standard solution (10 nmol/L). The porphyrins accumulated in the cells and in the medium were identified after separation by high performance liquid chromatography (HPLC). Uroporphyrin isomers were also separated by C18 reversed HPLC.

RESULTS

Transduction of target cells. Lymphoblastoid cell lines from patients with CEP were transduced with LXSN (control virus) and LUSN particles at a multiplicity of infection (MOI) of 2:1.

A Southern blot analysis showed that LB-deficient cell lines transduced with LUSN and selected by G418 carried 1 copy per genome. We observed the same gene transfer efficiency for the cells transduced by cocultivation and for those transduced by retroviral filtered supernatant (Fig 2). UROIIIS activity in normal cells as well as in transduced and nontransduced deficient cell lines. LB-, nontransduced LB-, and transduced LB- cells were collected before and after G418 selection for UROIIIS assay. UROIIIS activity was not detectable in nontransduced LB- or in LXSN-transduced LB- cells (Table 1 and data not shown). In contrast, after transduction of the LB- cells by LUSN, UROIIIS activity was increased to 20% and 24% of normal levels for the supernatant and the cocultivation techniques, respectively. The same result was observed during 12 weeks of culture, suggesting a stable integration and expression of the retroviral construct. A higher level of UROIIIS activity was
observed when the transduced cell lines were selected in medium containing G418 (115% and 127% of normal value, respectively; Table 1). Note that the level of UROIII activity of LB- cells transduced by coculture was not significantly different compared with LB- cells transduced by viral supernatant.

**Porphyridin accumulation.** To evaluate the ability of LUSN transduction to correct the metabolic defect, the profile of porphyrin accumulation was first studied in normal and deficient LB cells in the presence or absence of ALA, the natural precursor of tetrapyrrole biosynthesis. In the normal cell lines studied, a very low amount of porphyrin could be found when they were incubated in the absence of ALA. The level of porphyrins generated by normal cells in the presence of ALA was also very low and identified to be the natural precursor of tetrapyrrole biosynthesis. The different LB cells studied (Fig 4). For instance, when 50% of LB cells were cocultured with 50% of LB transduced cells or 50% of LBN cells, porphyrin accumulation was approximately half that of the accumulation corresponding to 100% of LB cells. These results demonstrate that there is no metabolic cross-correction of deficient cells by normal cells or by LB- transduced cells in vitro.

### DISCUSSION

Congenital erythropoietic porphyria is the most severe form of porphyria in humans. Forms of therapy that have
been described include hypertransfusion, splenectomy, the administration of haematin, oral activated charcoal, hydroxyurea, and low doses of chloroquine. A single patient has been treated by bone marrow transplantation from an HLA-identical sibling. There was a remarkable amelioration of the porphyria, but the patient died 11 months later from cytomegalovirus infection. Gene therapy by retrovirus-mediated insertion of the normal UROIIS gene into hematopoietic cells seems to be an appropriate treatment of the disease in the future. We constructed a recombinant retroviral vector LUSN to mediate insertion and expression of human UROIIS cDNA in a lymphopoietic target cell population. Lymphoblastoid cell lines from a patient with CEP were targeted for LUSN transduction.

A full restoration of enzymatic activity was found in transduced and G418-selected deficient cells. Also, a complete metabolic correction of the porphyric phenotype was accomplished, as indicated by a total reversion of porphyrin accumulation in the cells. Because we know that the efficiency of retroviral transduction of hematopoietic cells in vitro and in vivo is low, we wanted to look at the possibility of metabolic cross-correction between nontransduced and transduced deficient or normal cell lines. This metabolic correction in neighboring nontransduced cells is well-established for human lysosomal storage diseases such as mucopolysaccharidosis type II (Hunter syndrome) or type VII (Sly disease). Our experiments show the absence of cross-correction between nontransduced and transduced cells. This result is not surprising because we could expect an absence of transfer of the enzyme from one cell to the other and also because uroporphyrin I and coproporphyrin I, which are the accumulated porphyrins, correspond to a metabolic dead-end (Fig 5). Except for the case of a selective advantage of corrected cells, these results suggest that gene replacement need to be performed on a high percentage of cells for a substantial improvement of the biochemical abnormalities and a subsequent amelioration of the clinical manifestations. Because the accumulation of porphyrins predominates in the erythropoietic lineage, the ideal target cell for gene therapy of CEP should be the myeloid stem cell or the pluripotent stem cell for long-term therapy. Recent studies using stromal cell support or cytokines have shown an enhanced efficiency of viral gene transfer (including retrovirus and adenovirus-associated virus) into such cell populations selected for expression of the CD34 antigen and thus enriched for the reconstituting stem cell compartment. More studies with other enhancers (ie, the locus control region) and the use of hematopoietic progenitor cells are required for the achievement of high expression of the UROIIS cDNA in the erythropoietic lineage. However, we have documented sufficient gene transfer rate and metabolic correction in lymphopoietic cell lines to indicate that CEP should be a good candidate for treatment by gene therapy of hematopoietic stem cells.

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