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12. This is an adaptation (*6*) of a published procedure [D. M. Helfman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 31 (1983)]. The bacterial colonies were grown on a nitrocellulose filter; the filter-containing plates were kept inverted over 0.2 ml of chloroform for about 15 minutes. The filters were then peeled off and submerged in a solution containing saline (0.15*M* NaCl, 50 m*M* tris, *p*H 7.5), 3 percent bovine serum albumin (BSA), 1 m*M* $MgCl_2$, 1 μg of deoxyribonuclease and 40 μg of lysozyme per milliliter, and 0.1 m*M* phenylmethylsulfonyl fluoride for 60 minutes at room temperature. The filters were rinsed in saline and then incubated in 3 percent BSA-saline containing ^{125}I-labeled monoclonal antibody 2A10 (5×10^5 cpm/ml; specific activity 2×10^7 cpm per microgram of protein) at room temperature for 3 hours. The unbound radioactivity was washed off in saline plus 0.1 percent NP40 at room temperature, and the filters were mounted for autoradiography. Presumptive positive clones were picked, expanded, recloned, replicated, and retested with monoclonal antibody 2A10 and other monoclonal antibodies of the same isotype but of different specificity. The purification of monoclonal antibodies and the procedures for labeling were as described [see (*3*)].
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Expression of a Retrovirus Encoding Human HPRT in Mice

Abstract. *Transmissible retroviruses encoding human hypoxanthine phosphoribosyltransferase (HPRT) were used to infect mouse bone marrow cells in vitro, and the infected cells were transplanted into mice. Both active human HPRT-protein and chronic HPRT-virus production were detected in hematopoietic tissue of the mice, showing transfer of the gene. These results indicate the possible use of retroviruses for somatic cell therapy.*

The unique structure and mode of propagation of retroviruses make them ideally suited for gene transfer. These features include efficient transmission to recipient cells, integration into host chromosomal DNA, plasticity of the viral genome for accommodation of foreign DNA, and a wide cell type and host range. We have described the construction of an infectious retrovirus that contains the coding sequences for human hypoxanthine phosphoribosyltransferase (HPRT; E.C. 2.4.2.8) as a model system (*1*). In this vector (pLPL), a complementary DNA (cDNA) copy of the human gene coding for HPRT is expressed under the transcriptional control of viral long terminal repeats (LTR's). Several investigators have used similar vectors to transfer other genes into cultured cells (*2*). We now show that both enzymatically active human HPRT-protein and production of HPRT-virus can be detected in hematopoietic tissue of mice receiving transplants of bone marrow cells infected with HPRT-virus.

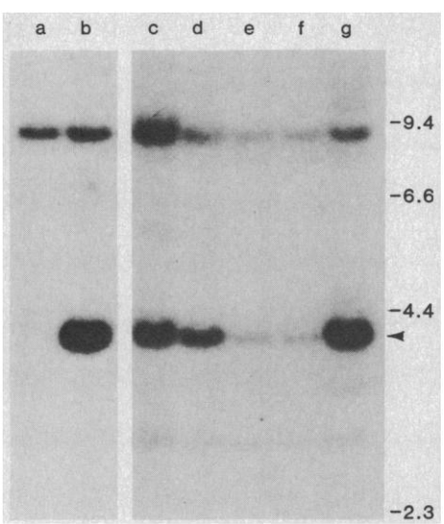

Fig. 1. Analysis of DNA from cultured cells converted to $HPRT^+$ by exposure to tissues from infected mice. $HRPT^+$ colonies induced by exposure to tissues of infected mice were propagated in bulk, and the DNA was extracted and analyzed by Southern transfer (*12*) with the use of an isotopically labeled probe made from a Pst I–Hind III fragment of the HPRT cDNA. (Lane a) Uninfected $HPRT^-$ cells; (lanes b and g) cultured cells infected with the virus used to infect the mice (c7c1); (lane c) DNA from $HPRT^+$ cells induced by exposure to spleen cells from a mouse 41 days after it was injected with bone marrow cells; (lane d) DNA from $HPRT^+$ cells induced by bone marrow cells from the same mouse; and (lanes e and f) DNA from $HPRT^+$ cells induced by spleen cells from two different mice 133 days after they were injected with bone marrow cells.

Mouse bone marrow cells were infected with HPRT-virus either by cocultivation with virus-producing cells or by direct infection with high-titer virus. Helper virus [Moloney strain (MoMLV)] was present in all infections so that rescue of replication-defective HPRT-virus could be monitored in recipient animals. We chose to infect bone marrow cells because pluripotent, self-renewing hematopoietic stem cells in bone marrow are probably among those cells that can be infected (*3*) and because bone marrow cells are readily accessible and have a high proliferation rate after reintroduction into mice. The infected bone marrow cells were then injected into irradiated mice of the same strain as the donor mouse. The radiation dose was chosen so that transplantation of fresh bone marrow was a requirement for survival of the mice. Thus, certain hematopoietic cellular compartments (bone marrow and spleen) depleted by irradiation were repopulated by the virus-infected bone marrow cells.

Spleen and bone marrow cells from mice receiving infected bone marrow were analyzed for production of HPRT-virus. It was expected that some hematopoietic cells from the mice receiving infected bone marrow would be infected with both the HPRT-virus and helper virus and thus would produce HPRT-virus. Spleen and bone marrow cells from mice receiving bone marrow transplants were removed and overlaid on adherent $HPRT^-$, ouabain-resistant cultured mouse cells (*4*). One day later the dishes were washed thoroughly to remove unattached cells and the remaining attached cells were exposed to selective medium containing HAT (see legend to Table 1) and ouabain. This selection ensures that only cultured cells converted to $HPRT^+$ by virus will survive because ouabain kills the donor mouse cells and HAT selects against $HRPT^-$ cells. The development of colonies of cells surviving HAT selection is thus dependent on production of HPRT-virus by the donor

cells. HPRT-virus was produced by spleen and bone marrow cells of mice infected with the high-titer HPRT-virus even at 133 days after transplantation, thus demonstrating long-term persistence of the HPRT-virus in these mice (Table 1).

DNA from cultured cells converted to HPRT$^+$ by exposure to spleen or bone marrow from mice receiving bone marrow transplants was analyzed for the presence of HPRT-viral DNA to determine whether the HPRT-virus was responsible for colony formation and that no rearrangement of the virus had occurred. Cellular DNA was digested with Sst I, subjectd to electrophoresis, and analyzed with an isotopically labeled HPRT probe. Sst I cuts in both LTR's of the virus; thus a single band of about 3.9 kilobases (kb) should be present in all infected cells. A probe specific for HPRT hybridized to a DNA species of about 3.9 kb (Fig. 1, lanes b and g) from cultured cells infected with stock HPRT-virus, and no hybridization to a DNA of similar size occurred in uninfected cells (lane a). An additional band of about 9 kb representing the endogenous mouse HPRT DNA can also be seen in both samples. The 3.9-kb band representing the HPRT-viral DNA was also seen in cultured cells converted to HPRT$^+$ by exposure to cells from infected mice (Fig. 1, lanes c to f). Thus HPRT-virus released from mouse tissue was identical to that originally used for infection of the mice.

In another experiment, proteins from spleen and bone marrow of several mice were analyzed for the presence of human HPRT 2 months after bone marrow transplantation. We were able to detect human HPRT in the spleen of one animal. Two HPRT isoenzymes were present in normal mouse spleen (Fig. 2, lane a), and three major isoenzymes were present in fetal human spleen (lane b). In the spleen from the mouse receiving infected bone marrow (lane c), a form of HPRT that migrates along with the upper human isoenzyme was visible in addition to the normal mouse isoenzymes. After longer exposure of photographic film to the radioactive gel, a band migrating with the middle human isoenzyme was also detected.

It has been shown that HPRT activities resolved in this gel system are due to HPRT dimers and that heterodimers can form between human and mouse HPRT in hybrid cells expressing both genes (*5*). A putative heterodimer band was present between bands representing fetal human and mouse spleen protein in spleen protein from the mouse receiving infected bone marrow (Fig. 2, arrow), implying that human and mouse HPRT were synthesized in the same cells. These heterodimers were not seen after mixtures of human and mouse cell lysates were subjected to electrophoresis (*5*) (data not shown). We also analyzed the HPRT-virus–producing rat fibroblasts used for bone marrow infection (lane d). These cells expressed human HRPT isoenzymes with mobilities similar to those of fetal human spleen (lane g) but in different proportions. No rat HPRT activity was detected because the parental rat cell line was HPRT$^-$. Normal rat fibroblasts displayed a pattern different from all others (lane e), showing that protein expressed by the HPRT-virus was not of rat origin.

Lesch-Nyhan syndrome is apparently

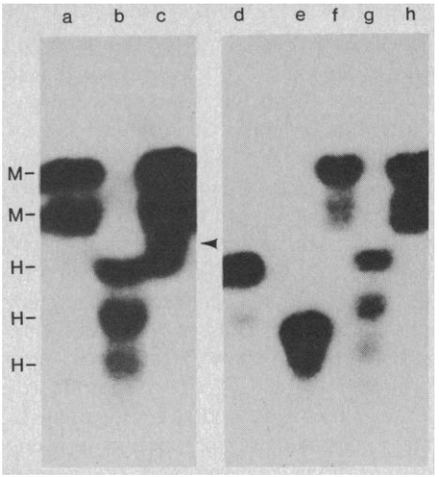

Fig. 2. Analysis of mouse hematopoietic tissue for the presence of human HPRT. Bone marrow cells from the spine of a C57BL/6 mouse were removed (*13*) and portions of nucleated cells (5×10^7) were overlaid in 10-cm dishes of confluent 208F rat cells, which produce HPRT-virus (5×10^3 per milliliter per 16 hours) and MoMLV helper virus (5×10^4 per milliliter per 16 hours) (*1*). Viral infections were performed in Dulbecco-Vogt modified Eagle's medium containing 8 μg of Polybrene (Sigma) per milliliter and 10^{-5} *M* 2-mercaptoethanol. After 16 hours nonadherent cells were removed and resuspended at 2×10^7 viable cells per milliliter (85 percent of the cells were viable as judged by trypan blue dye exclusion), and 0.5-ml portions were injected intravenously into C57BL/6 recipient mice that had earlier received 1200 rads from a ^{60}Co source. Two months after bone marrow injection, mouse tissue was analyzed for the presence of human HPRT activity after isoelectric focusing (*7*). (Lanes a, f, and h) Protein from the spleen of an irradiated mouse receiving mock-infected bone marrow cells; (lanes b and g) protein from fetal human spleen; (lane c) protein from spleen of a mouse receiving HPRT-virus–infected bone marrow cells; (lane d) protein from the 208F rat cells used to produce the HPRT-virus; and (lane e) protein from normal rat fibroblasts. M denotes mouse HPRT, and H denotes human HPRT isoenzymes. The arrow in lane c indicates the putative mouse-human heterodimer.

Table 1. Virus production and persistence in mouse spleen and bone marrow. Bone marrow cells from the spine of a C57BL/6 mouse were isolated (*13*) and infected with HPRT-virus harvested from a cell line [pLPL/pSAM c7cl (*14*)] that overexpresses HPRT-virus. Medium harvested after 16 hours from c7cl cells contained 2×10^7 HPRT-virus per milliliter and less than 10^4 helper virus per milliliter. Nucleated bone marrow cells (5×10^8) were exposed to HPRT-virus in two 1-hour incubations (100 ml of virus each) at room temperature in the presence of 8 μg of Polybrene (Sigma) per milliliter and 10^{-5} *M* 2-mercaptoethanol. After each incubation, the cells were centrifuged at 600*g* for 10 minutes and the medium was removed. The cells were suspended in 10 ml of medium containing 10^7 MoMLV helper virus, and 0.5-ml doses were injected into the tail veins of mice previously exposed to 1200 rads from a ^{60}Co radiation source. Spleen and bone marrow (spine) cells were isolated at the time indicated and assayed for virus production. HPRT-virus assay was performed by overlay of 10^7 mouse cells on HPRT$^-$, ouabain-resistant BALB/3T3 cells (5×10^5) (*4*) in a 5-cm dish. After 16 hours the dishes were washed three times to remove nonadherent mouse cells. The cells were trypsinized and divided 1:5 into medium containing 30 μ*M* hypoxanthine, 1 μ*M* amethopterin, 20 μ*M* thymidine (HAT medium), and 5 m*M* ouabain. Colonies were counted 6 to 10 days later. MoMLV helper virus was measured by the XC plaque assay (*15*). Briefly, 10^7 mouse cells were overlaid on NIH 3T3 TK$^-$ cells (5×10^5) for 16 hours in a 5-cm dish. The dishes were washed three times and the cells were divided 1:10. Three days later the cells were exposed to ultraviolet radiation and overlaid with XC cells. Two days later, the cells were stained and plaques were counted. Titers of MoMLV were approximately 10^6 XC plaque-forming units per 10^7 cells per 16 hours.

Mouse tissue	Time after transplant (days)	HPRT$^+$ colonies (CFU per 10^7 cells per 16 hours)*
	Control	
Spleen	31	<5
Bone marrow	31	<5
	Infected	
Spleen	31	90
Bone marrow	31	40
Spleen	41	340
Bone marrow	41	200
Spleen	41	540
Bone marrow	41	125
Spleen	113	480
Bone marrow	113	720
Spleen	133	230
Spleen	133	160
Spleen	133	330

*HPRT$^+$ colony-forming units (CFU) produced by 10^7 cells during overlay for 16 hours on recipient cells.

caused by a defect in the HPRT gene (*6*). One of the goals of our research is to test the utility of retroviral vectors as potential agents for correction of this defect in somatic cells. We have shown that lymphoblasts from Lesch-Nyhan patients can be infected with the HPRT-virus in vitro (*7*). Results presented here show that the HPRT-virus can be transferred into intact animals because (i) bone marrow and spleen cells of mice receiving infected bone marrow became long-term producers of HPRT-virus, and the released virus appeared to be identical to the virus used for infection of the engrafted bone marrow and (ii) human HRPT was detected in the spleen of a recipient mouse.

From these results we cannot estimate the number of hematopoietic cells infected by HPRT-virus. Because we could detect HPRT-protein clearly in only one animal, transfer may have been relatively inefficient. However, several lines of evidence suggest that $HPRT^+$ hematopoietic cells have a selective advantage over $HPRT^-$ hematopoietic cells. The tissue distribution of $HPRT^-$ cells in partially $HPRT^-$ mosaic mice suggests that selection against $HPRT^-$ cells is particularly strong in blood (*8*). A similar conclusion was made in studies of human heterozygotes carrying the defective HPRT gene (*9, 10*). In addition, myeloid progenitor cells from patients with HPRT deficiency grow poorly in culture compared with those from normal patients (*11*). These results suggest that, even though the HPRT-virus may not be expressed in every cell in virus-treated marrow, retroviral transfer of the HPRT gene into somatic cells from Lesch-Nyhan patients may be effective because of the presumed selective advantage conferred by the synthesis of HPRT in some infected cells.

Note added in proof: Expression of human HPRT protein activity has also been detected in the spleen of another mouse transplanted with amphotropic HPRT-virus obtained from the cell clone c7cl.

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Characterization and Localization of Proopiomelanocortin Messenger RNA in the Adult Rat Testis

Abstract. *Northern blot analysis of total RNA and polyadenylated RNA isolated from adult rat testes showed that a proopiomelanocortin (POMC)-like messenger RNA molecule is present in these extracts. The testicular POMC messenger RNA is comparable in length to amygdala and midbrain POMC messenger RNA and appears to be at least 200 nucleotides shorter than POMC messenger RNA found in the hypothalamus and anterior and intermediate lobes of the pituitary gland. Hybridization in situ showed that POMC messenger RNA is located in Leydig cells, which are the only testicular cells that contain immunostainable POMC-derived peptides. These results suggest that local synthesis of POMC occurs in the testis.*

The potential physiological role for peptide hormones originally isolated from the pituitary gland has increased recently with numerous demonstrations that these hormones are present in a number of mammalian nonpituitary tissues (*1*). For example, peptides related to proopiomelanocortin (POMC) have been identified immunocytochemically at various sites in the reproductive tract of the adult male rat, including the Leydig cells of the testis (*2*). Electrophoretic and chromatographic techniques, combined with radioimmunoassay, have been used to determine the molecular species and concentrations of POMC-related peptides in the testis (*3*). Fragments of sizes corresponding to those of endorphin and α-melanocyte–stimulating hormone (α-MSH) are the major POMC-derived peptides present in adult testis extracts; however, in contrast to those found in the adult rat intermediate pituitary lobe (*4*), these testicular POMC-derived peptides are present in their nonacetylated forms (*3*). These results show that the end points of POMC processing in the reproductive tract are similar to those in the hypothalamus, where nonacetylated forms of these peptides are also present (*5*). The concentrations of POMC-related peptides present in the adult rat testis are not altered by hypophysectomy (*3*), suggesting that testicular POMC peptides are not derived from pituitary secretion and subsequent uptake by testicular cells. This observation, however, does not eliminate the possibility that these peptides may be synthesized elsewhere.

Complementary DNA (cDNA) probes can be used to determine whether messenger RNA (mRNA) that can direct local synthesis of specific peptides or proteins is present in specific tissues. Thus far, POMC cDNA probes have been used to demonstrate POMC-like mRNA in specific brain regions (*6*) and to locate cells containing POMC-like mRNA in the periarcuate region of the hypothalamus (*7*). We have investigated whether a cDNA probe for POMC can detect POMC-like mRNA in testis extracts and whether this probe can locate specific cells containing POMC mRNA after hybridization in situ.

RNA was isolated from adult male pituitary, liver, testis, amygdala, and midbrain by the guanidinium isothiocyanate procedure (*8*); polyadenylated