exon L2 in that /CUCGCG was replaced by /CUGCAGGUCGAC, thereby creating Pst I and Sal I restriction sites in the exon. For both the full-length AdML and AdML/5′SS-BS substrates, the 5′ exon, 5′ splice site (/), and intron sequences up to the branch point (**A**) are:

```
5′-GGGAGACCGGCAGAUCAGCUUGGCCGCGUCCAUCUG
UCAUCUAGUGAUAUCAUCGAUGAAUUCGAGCUCGGUACC
CCGUUCGUCCUCACUCUCUUCCGCAUCGCUGUCUGCGAG
GGCCAGCUGUUGGG/GUGAGUACUCCCUCUCAAAAGCGG
GCAUGACUUCUGCCUCGAGUUAUUAACCCUCACUAAAG
GCAGUAGUCAAGGGUUUCCUUGAAGCUUUCGUGCUGAC-3′
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In Figs. 2B, 2C, 3, and 4, a truncated version of the 5′ substrate was used in which nucleotides 5 to 46 of the 5′ exon had been deleted. The TNT exon sequence is identical to the optimized exon 5 mutation N of chicken cardiac troponin T (*20*). Full-length AdML RNA was transcribed with T7 polymerase from pAdMLpar that had been linearized with Bam HI. All other RNAs were transcribed from T7 templates generated by PCR with either self-complementary primers (TNT 3′ substrates) or primers complementary to either pAdMLpar (AdML 3′ substrates) or pAdMLDAG (AdML/5′SS-BS RNA). RNAs were labeled internally with either [α-^{32}P]ATP (adenosine triphosphate) or [α-^{32}P]UTP (uridine triphosphate). All AdML/5′SS-BS and 3′ substrate RNAs in Figs. 1B, 2A, 2C, and 4 contained G(5′)ppp(5′)G caps. In Figs. 2B, 3A, and 3B, the 3′ substrate RNAs were capped with GMP (guanosine monophosphate).

31. Splicing reactions were performed at 30°C in volumes of 5 or 10 μl containing 40% (w/v) HeLa nuclear extract (HeLa cells were obtained from Cellex Biosciences, Minneapolis, MN), 70 to 85 mM KCl, 2 mM $MgCl_2$, 1 mM ATP, and 5 mM creatine phosphate. When present, the 5′ substrate was 35 nM and the 3′ substrates were 175 nM. RNAs were extracted and separated by denaturing polyacrylamide gel electrophoresis. All gels were subjected to autoradiography and quantitated with a Molecular Dynamics PhosphorImager.
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33. The slight fuzziness of the bands in Figs. 2B and 3B was likely due to heterogeneity at the 3′ end of the 3′ substrate. Blurred bands were observed only in experiments with the truncated version of the 5′ substrate and AdML 3′ substrates separated on low-percentage gels (8 to 10% polyacrylamide). The AdML 3′ substrate terminates at a Bam HI restriction site (Fig. 1A), which is known to cause extensive 3′ end heterogeneity in run-off transcripts [for example, see figure 2B, in M. J. Moore and P. A. Sharp, *Science* **256**, 992 (1992) and references therein].
34. We thank R. Reed for plasmids pAdMLpar and pAdMLDAG; T. Cooper and A. Zahler for cTNT sequences; and L. Davis, J. Gelles, C. Miller, C. Query, M. Rosbash, and P. Sharp for critical reading of the manuscript. This work was supported by NIH grant GM53007, a Packard Fellowship, and a Searle Scholarship.

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Trans-kingdom Transposition of the *Drosophila* Element *mariner* Within the Protozoan *Leishmania*

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Transposable elements of the *mariner*/Tc1 family are postulated to have spread by horizontal transfer and be relatively independent of host-specific factors. This was tested by introducing the *Drosophila mauritiana* element *mariner* into the human parasite *Leishmania major*, a trypanosomatid protozoan belonging to one of the most ancient eukaryotic lineages. Transposition in *Leishmania* was efficient, occurring in more than 20 percent of random transfectants, and proceeded by the same mechanism as in *Drosophila*. Insertional inactivation of a specific gene was obtained, and a modified *mariner* element was used to select for gene fusions, establishing *mariner* as a powerful genetic tool for *Leishmania* and other organisms. These experiments demonstrate the evolutionary range of *mariner* transposition in vivo and underscore the ability of this ubiquitous DNA to parasitize the eukaryotic genome.

Transposons of the *mariner*/Tc1 family are ubiquitous elements of eukaryotic genomes, occurring in virtually every taxon examined (*1*–*3*). Phylogenetic studies of *mariner* elements have provided compelling evidence for the occurrence of horizontal transfer across species during evolution, traversing distances as far as that separating insects and flatworms (*1*, *2*, *4*). This suggested that *mariner* could transpose independently of host-specific factors, a belief bolstered by studies of transposition activity in vitro (*5*). Hence, *mariner* was advanced as a potentially general tool for stable transformation and insertional mutagenesis in eukaryotic genomes after heterologous expression (*2*, *3*, *6*, *7*). However, thus far this prediction has only been fulfilled in transfers among relatively closely related species within the order Diptera, as seen with the *Drosophila* elements *mariner*, *hermes*, *hobo*, and *minos* (*7*–*9*).

We decided to probe the evolutionary limits of *mariner*'s ability to transpose in vivo by introducing it into *Leishmania major*, a human pathogen belonging to the flagellate order Kinetoplastida, one of the earliest branching eukaryotic lineages (*10*). Success here could also provide genetic methods to study processes of virulence and pathogenesis in leishmaniasis, a widespread tropical disease that can frequently be fatal and for which satisfactory vaccines or chemotherapy are lacking. Although methods for stable DNA transfection and expression of foreign genes are well established in *Leishmania*, nonhomologous insertion of DNA has not been observed in stable transfections of this diploid organism (*11*). Mobilization of *mariner* would thus provide a powerful tool for insertional mutagenesis in this pathogen.

The 1.3-kb *Mos1 mariner* element from *Drosophila mauritiana* contains a single open reading frame (ORF) encoding the transposase, flanked by 28–base pair (bp) inverted repeats (*12*). An intact *mariner* element was inserted in one *Leishmania* vector (pX63PAC-Mos1, Fig. 1A) (*13*), and a helper plasmid was used to provide transposase. *Leishmania* and other trypanosomatid protozoa synthesize mRNAs by a trans-splicing mechanism, where a 39-nucleotide mini-exon is added to the 5′ end of every mRNA (*14*). Accordingly, the *mariner* transposase coding region was inserted in a *Leishmania* expression vector (pX63TKNEO-TPASE, Fig. 1A) (*13*) downstream of a trans-splice acceptor site because *Drosophila* genes lack these RNA signals. The two plasmids were then introduced into *Leishmania major* line +/Δ1 (*15*). *Leishmania* plasmids are maintained as stable episomes while under drug pressure (G418 and puromycin for the *NEO* and *PAC* markers, respectively) but are slowly lost during growth in the absence of selection (*16*).

Transfectant colonies were analyzed for *mariner* transposition first by Southern (DNA) blot hybridization (*17*). Despite the lack of any selection for transposition, 5 of 22 colonies (23%) showed new *mariner*-hybridizing bands (Fig. 1B). No evidence of transposition was obtained in Southern blot analysis of 52 colonies containing only pX63PAC-Mos1 (*18*). The *mariner* insertion site from several of the new fragments arising in the presence of transposase was obtained by inverse polymerase chain reaction (PCR) (*19*). Sequence analysis showed that they contained *mariner*, followed by a TA dinucleotide and sequences not present in the donor plasmid DNA (Fig. 1C) (*19*). Southern blot hybridizations with the new *mariner*-flanking sequences showed that they were of *Leishmania* origin (*20*). Moreover, their fragment size had increased by 1.3 kb in the colony that gave rise to the PCR product (*20*), as expected for bona fide transposition.

The frequency of *mariner* insertion into a specific locus was measured for dihydrofolate reductase–thymidylate synthase (*DHFR-TS*). The parental +/Δ1 line used in the studies above is heterozygous, having one copy of

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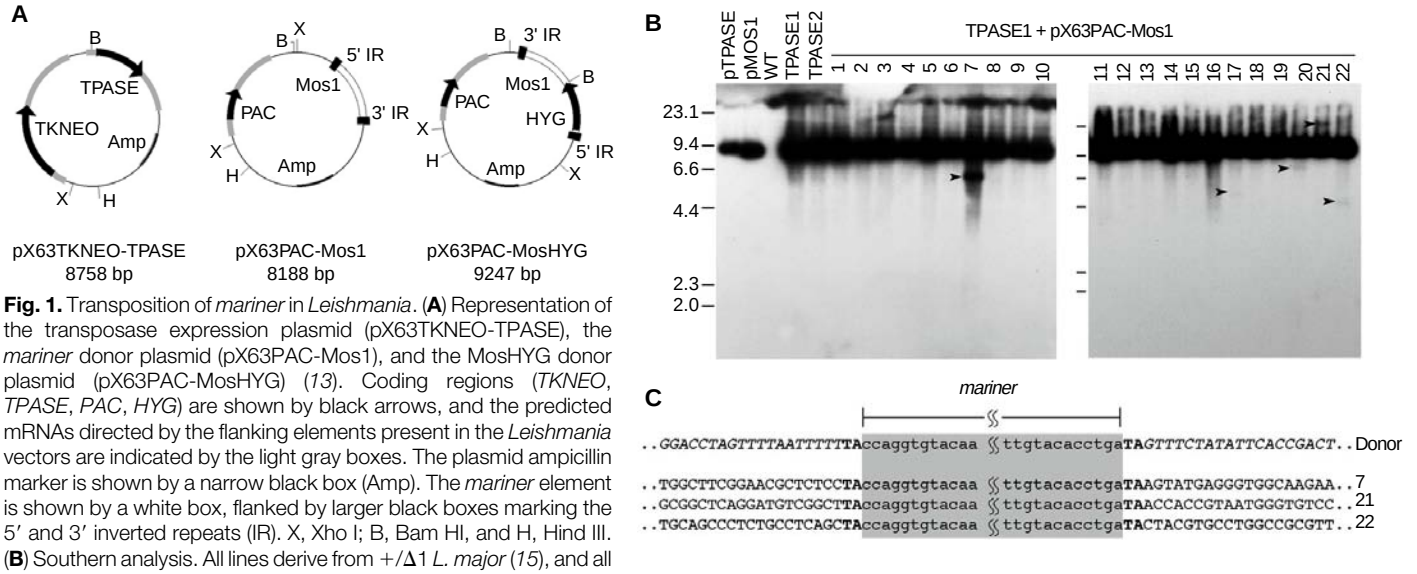

Fig. 1. Transposition of *mariner* in *Leishmania*. (**A**) Representation of the transposase expression plasmid (pX63TKNEO-TPASE), the *mariner* donor plasmid (pX63PAC-Mos1), and the MosHYG donor plasmid (pX63PAC-MosHYG) (*13*). Coding regions (*TKNEO*, *TPASE*, *PAC*, *HYG*) are shown by black arrows, and the predicted mRNAs directed by the flanking elements present in the *Leishmania* vectors are indicated by the light gray boxes. The plasmid ampicillin marker is shown by a narrow black box (Amp). The *mariner* element is shown by a white box, flanked by larger black boxes marking the 5′ and 3′ inverted repeats (IR). X, Xho I; B, Bam HI, and H, Hind III. (**B**) Southern analysis. All lines derive from +/Δ1 *L. major* (*15*), and all DNAs were digested with Hind III. Lanes: pTPASE, pX63TKNEO-TPASE DNA; pMos1, pX63PAC-Mos1 DNA; WT, +/Δ1 genomic DNA; TPASE1 and TPASE2, genomic DNA from independent pX63TKNEO-TPASE–transfected lines; TPASE1 + pX63PAC-Mos1, genomic DNA from 22 independent colonies containing both plasmids pX63TKNEO-TPASE and pX63PAC-Mos1. Arrows point to new *mariner* bands; differences in intensity may reflect transposition at various times during colony growth. The *mariner* hybridization probe was the 1.1-kb Cla I–Nhe I fragment from pBluescribe M13+/Mos1 (*12*). Size markers are in kilobases. (**C**) DNA sequence of *mariner* transpositions. Inverse PCR (*19*) was used to recover the new fragments evident in clones 7, 21, and 22 [(B), arrows], and their DNA sequence was determined. The *Drosophila* sequences present in the *Mos1* donor plasmid are in italics, the *mariner* sequence is in the central gray box, and the TA dinucleotides marking the boundary between *mariner* and *Leishmania* sequences are in bold type. Because 5′ and 3′ sequences were obtained independently, they possibly may not arise from the same insertion event.

DHFR-TS over a deletion allele, and we have shown previously (*21*) that one can select against *DHFR-TS* by plating these parasites in the presence of methotrexate (MTX) and thymidine (TdR). Surviving parasites undergo either loss-of-heterozygosity (LOH) or inactivating point mutations, and we anticipated that *mariner* insertion into *DHFR-TS* would similarly inactivate it.

Plating of *mariner*-containing strains on MTX plus TdR yielded MTX-resistant (MTX^r) colonies at a frequency of 1.2×10^{-4}, as reported previously (*21*). Southern blot analysis showed that 39 colonies exhibited LOH, and 9 colonies retained a presumably altered *DHFR-TS* allele [Fig. 2A or (*20*); line 7M9 shows LOH and the rest retain *DHFR-TS* sequences]. Of the non-LOH colonies, one (22M3) exhibited a *DHFR-TS* fragment of 7.1 kb, 1.3 kb larger than the wild-type 5.8-kb fragment (Fig. 2A), and rehybridization of the blot with a *mariner* probe identified the same 7.1-kb fragment (Fig. 2B). Sequencing of the 22M3 *mariner* insertion confirmed that it had transposed into *DHFR-TS*, into a TA dinucleotide located at position 532 within the *DHFR* coding region, and led to duplication of the target TA (Fig. 2C). Together with the results shown in Fig. 1C, it is evident that transposition in *Leishmania* proceeds by the characteristic mechanism of the *mariner*/Tc1 family, involving insertion into and duplication of an invariant target TA dinucleotide (*22*).

Several of the random colonies lacking insertions in *DHFR-TS* showed new *mariner*-hybridizing fragments (7M8, 7M9, and 17M11; Fig. 2B), suggestive of transposition events involving other unselected loci within the *Leishmania* genome. Because of the discontinuation of G418 and puromycin selection, the parental plasmids were lost in some colonies (22M8 and 22M10; Fig. 2B).

An estimate for the frequency of *mariner* transposition in *Leishmania* was calculated, $(1/48) \times (1.2 \times 10^{-4})$, or about 2.5×10^{-6} for the insertional inactivation of a single allele. Because *DHFR-TS* spans 1.5 kb, and the *Leishmania* genome is about 50,000 kb and diploid, this leads to an estimated frequency for independent *mariner* transpositions of more than 10% per genome. This is in good agreement with the results obtained from Southern blots of random colonies (Figs. 1B and 2B). Future studies may permit more accurate measurement of the rate of transposition and the development of methods for increasing or regulating it.

We asked whether a modified *mariner* element carrying a drug resistance marker could

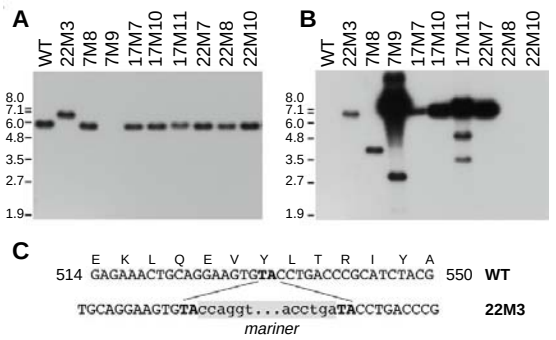

Fig. 2. Insertional inactivation of *DHFR-TS* by *mariner*. (**A**) Southern blot analysis of the *DHFR-TS* locus from colonies obtained after selection against DHFR-TS expression by plating of lines 7, 17, and 22 (Fig. 1B) on MTX plus TdR. WT, +/Δ1; the remaining lines are named after the original colony and the specific independent clone obtained after selection. Thus, line 22M3 is the third colony examined after plating of line 22. DNAs were digested with Eco RV and Hind III, and the probe was a Bgl II–Sty I 0.7-kb fragment of the coding region of the *Leishmania donovani DHFR-TS* gene (clone pDDHFR8; strain B2064). Size markers are in kilobases. (**B**) The probe from the blot in (A) was removed, and the blot was rehybridized with the *mariner* probe (see legend to Fig. 1B). In some colonies, the parental donor plasmid was retained. (**C**) Sequence of the *mariner* insertion in mutant 22M3 and comparison with the wild-type *DHFR-TS* sequence. The nucleotide coordinates for the *DHFR-TS* coding region are shown flanking the sequence, and the amino acid sequence is shown above. The TA insertion site and its transposition-generated duplication in line 22M3 are shown in bold. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; E, Glu; I, Ile; K, Lys; L, Leu; Q, Gln; R, Arg; T, Thr; V, Val; and Y, Tyr.

be used to "trap" and thereby identify new genes within *Leishmania*. The MosHYG element contains a hygromycin resistance gene (*HYG*) inserted immediately adjacent to the *mariner* 28-bp inverted repeats, 5′ of the transposase (Fig. 1A). In this configuration, the *HYG* gene in MosHYG lacks *Leishmania* splice acceptor elements, contains several upstream in-frame stop codons, and is placed in an antisense orientation relative to mRNA processing signals present elsewhere in the pX63PAC vector (Fig. 1A). Thus, hygromycin resistance should only arise after transposition of MosHYG downstream of a *Leishmania* splice acceptor.

Transfections of 10 μg of pX63PAC-MosHYG DNA into cells containing pX63TKNEO-TPASE yielded 110 to 160 colonies when plated on puromycin media but no colonies when plated on hygromycin media, confirming that *HYG* is initially silent in the context of the donor plasmid. However, replating eight independent lines containing both plasmids on hygromycin media yielded colonies at frequencies ranging up to 10^{-3} (*23*). In contrast, selection of 21 cell lines containing only pX63PAC-MosHYG did not yield any hygromycin-resistant parasites, suggesting that *Leishmania* lacks an endogenous transposase activity.

Transpositional activation of *HYG* should generate chimeric mRNAs containing the *Leishmania* mini-exon sequence, a variable amount of intervening 5′ untranslated sequence, and the MosHYG element (Fig. 3A). Reverse transcriptase–PCR (RT-PCR) with mini-exon– and *HYG*-specific primers was used to recover the predicted mRNA in several lines, and sequence analysis revealed the expected chimeric structure (*24*) (Fig. 3A). These findings were confirmed by Northern (RNA) blot analysis. When a probe derived from transposition event T3.6B was used, a 4.4-kb mRNA was observed in wild-type parasites, or in parasites bearing different transpositions. In contrast, a 6.9-kb mRNA was additionally found in the parental line T3.6 (Fig. 3, A and B), and, as expected, the increase in size (2.5 kb) was close to the size of the MosHYG element (2.4 kb). Similar results were obtained with the T3.5B probe (*20*) (Fig. 3A).

Most of the trapped *Leishmania* sequences did not show matches in database searches, although one (T1.2D) showed insertion into the 5′ untranslated region of the *DHFR-TS* locus (Fig. 3A). Further analysis showed that in this colony, *mariner* had transposed into the donor plasmid pX63PAC-MosHYG, which contains *DHFR-TS* flanking sequences to drive expression of the *PAC* marker (*25*). Because the *DHFR-TS* splice acceptor site was used normally (*26*), and Northern blot analyses revealed the expected increases in mRNA size from insertion of the MosHYG elements, we conclude that *mariner* insertion does not interfere with processing of endogenous mRNAs.

In summary, we have shown that *mariner* can transpose efficiently in *Leishmania* and have used it as an insertional mutagen and to trap new *Leishmania* genes. Classical genetic studies of *Leishmania* as well as of other medically important organisms such as trypanosomes and several pathogenic fungi are hampered because these parasites are diploid and often lack an experimentally manipulable sexual cycle. Thus, reverse genetic approaches are particularly important. The development of a *mariner*-based heterologous transposon system should prove a significant addition to the array of tools available for dissecting important aspects of *Leishmania* biology, such as virulence and pathogenesis.

Our data reinforce the impression that *mariner* and related elements are autonomous and able to cross distant species boundaries in vivo. Many workers have suggested that *mariner* could be adapted for use as a wide-range transformation vector (*2*, *3*, *6*, *7*). Previously, heterologous *mariner*/Tc1 transposition had been observed, but only within members of the same taxonomic order (*7*, *8*). Our findings now extend the range to different kingdoms, separated by an evolutionary distance of probably more than 1 billion years. To our knowledge, this is the widest evolutionary distance yet shown to be traversed by transposable elements in vivo and suggests that *mariner*'s potential utility may be comparably broad. Thus, in addition to its role in shaping eukaryotic genomes (*3*, *27*), this parasitic DNA can now be applied toward probing the genomes of human parasites and, conceivably, many other eukaryotes.

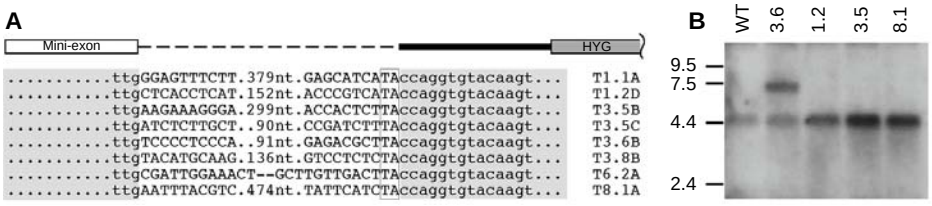

Fig. 3. Use of a modified *mariner* element to trap expressed regions of the *Leishmania* genome. (**A**) Sequence of RT-PCR products corresponding to transposition events generating chimeric *HYG*-containing RNAs (*24*). The numbers indicate the specific *Leishmania* line (1.1, 1.2, 3.5, 3.6, 3.8, 6.2, and 8.1) obtained after plating colonies bearing pX63TKNEO-TPASE and pX63PAC-MosHYG on hygromycin plates; the letters A to D denote the specific PCR amplification product sequenced. The structure of the predicted chimeric transcripts is shown above the sequences and consists of the mini-exon (open box on left), *Leishmania* sequences (dashed line), the 28-bp *mariner* inverted repeat (heavy black line), and the *HYG* resistance marker (gray box). For clarity, dashes were arbitrarily added to T6.2A, and the entire sequence of the products is not shown (nt, nucleotides). (**B**) Northern blot analysis of total RNAs from the +/Δ1 (WT) and several hygromycin^r lines described in (A). The hybridization probe was prepared by inverse PCR and corresponded to the T3.6B product (A), which arose from line 3.6 (*40*). Similar results were obtained with the T3.5B product, which arose from line 3.5 (*20*). Size markers are in kilobases.

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13. pX63TKNEO (strain B2179) and pX63PAC (B1129) are *Leishmania* expression vectors derived from pX63NEO (*28*) by replacing the *NEO*-containing Spe I fragment with the Bgl II–Sma I 2.2-kb fragment of pTNFUS69Py (*TKNEO*) (*29*) or the Not I–Nco I 0.7-kb fragment of pVN3.1 (*PAC*) (*30*). pX63TKNEO-TPASE (B3078) contains the 1.4-kb Cla I–Hind III fragment from pBluescribe M13+/Mos1 (*12*) inserted into the Sma I expression site of pX63TKNEO. This fragment starts 69 nucleotides before the transposase ATG and extends beyond the stop codon, including the 3′ inverted repeat and nearly 200 bp of *Drosophila* flanking DNA. pX63PAC-Mos1 (B3079) contains the 2.2-kb Xho I–Hind III fragment of pBluescribe M13+/Mos1, inserted into the Bgl II site of pX63PAC. pX63PAC-MosHYG (B3081) was constructed by blunt-end cloning of a 1.1-kb Spe I–Bam HI hygromycin phosphotransferase cassette from pX63HYG (*31*) into the Cla I site of *Mos1*. The sequence of the *Drosophila* DNA flanking *Mos1* has been deposited in GenBank (U96147, U96148).
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15. Transfections, plating on semisolid media, and drug selections were performed as described (*16, 32*). Drug concentrations were as follows: G418, 8 to 16 μg/ml; hygromycin B, 16 to 32 μg/ml; and puromycin, 25 to 50 μM. The *L. major* strain +/Δ1 was derived from the null-targeted *dhfr-ts*$^-$ line C1 (*21*) by

transfection with a wild-type *DHFR-TS* fragment [9-kb Cla I fragment of pK300 (*16*)] and selection for thymidine prototrophy. The +/Δ1 strain of *L. major* was initially transfected with pX63TKNEO-TPASE to give rise to line TPASE1. TPASE1 was subsequently transfected with pX63PAC-Mos1 in the presence of both G418 and puromycin. Selection against DHFR-TS expression was accomplished by plating cells on semisolid M199 medium containing TdR (10 μg/ml) and MTX (100 μM) (*21*) but lacking G418 or puromycin.
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17. *Leishmania* DNAs were prepared by the LiCl miniprep method (*33*) and used in standard Southern blot analysis (*34*) with probes prepared by the random priming method (*35*).
18. Several of these colonies did show higher molecular weight bands; however, their mobility was the same as higher molecular weight bands arising from the input transfecting DNA, and we attribute this to the presence of partial digestion of monomer or multimeric pX63PAC-Mos1, which is expected to occur in both *Escherichia coli* and *Leishmania* (*16, 36*).
19. We obtained the sequences shown in Fig. 1C by inverse PCR, using different pairs of primers for each side of the transposon. The 5′ side primers (as defined by the transposase ORF within the *Mos1* element) were B440 (5′-GCCGAACTGCAAGCATTATTGG; sense) and B441 (5′-TGAAGCGTTGAAACCACCGTTC; antisense); the 3′ side primers were B442 (5′-TCCACAAATTGCCCGAGAGATG; sense) and B443 (5′-ATGTGATGGAGCGTTGTCATGG; antisense). First, genomic DNAs were separated on agarose gels after restriction digestion, and DNA fragments in the size range for the new *mariner*-hybridizing band were isolated. These were then digested with Sau 3A, self-ligated under dilute conditions, and then used in standard PCR reactions (*37*). Products differing in size from those arising from the parental plasmids were identified by gel electrophoresis, inserted into pGEM-T (Promega), and sequenced with ΔTaq 2.0 polymerase and the Taquenase kit (United States Biochemical). Sequences were deposited in GenBank under the accession numbers B07506 through B07511.
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24. RNAs were prepared with Tri-reagent (Sigma) (*38*), following the procedures of the manufacturer. cDNA was synthesized from ~1 μg of total RNA, with a random hexamer priming protocol (*39*). A nested RT-PCR protocol was used to amplify chimeric *Leishmania*-MosHYG mRNAs (*39*). For the first PCR, the anchor primer was B466 (5′-AACGCTATATAAGTATCAG), corresponding to nucleotides 1 through 19 of the mini-exon, and the *HYG*-specific primer was B467 (5′-AAAGCACGAGATTCTTCG). Primers for the second round of PCR were B145 (5′-ATCAGTTTCTGTACTTTA; nucleotides 15 through 32 of the mini-exon) and the *HYG*-specific primer B456 (5′-ATCAGAAACTTCTCGACAG). RT-PCR products were cloned into pGEM-T and sequenced. Sequences were deposited in GenBank under the accession numbers AA195776 through AA195783.
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40. Northern blots were performed with glyoxal-denatured RNAs (*34*). The probes used in Northern analysis (Fig. 3B) were prepared by inverse PCR, using +/Δ 1 DNA digested with Sau 3A or Hpa II, followed by self-ligation under dilute conditions, as template; for sequence T3.5B, the primers were B523 (5′-CGGCTGCTCTGCTCTTCC; sense) and B524 (5′-TCTCTCGTGGCTCTGTGTACC); for sequence T3.6B, the primers were B525 (5′-CGCTTACATGCACGGTGC; sense) and B526 (5′-AACGGTGTCCACGACGGC).
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HSV-TK Gene Transfer into Donor Lymphocytes for Control of Allogeneic Graft-Versus-Leukemia

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In allogeneic bone marrow transplantation (allo-BMT), donor lymphocytes play a central therapeutic role in both graft-versus-leukemia (GvL) and immune reconstitution. However, their use is limited by the risk of severe graft-versus-host disease (GvHD). Eight patients who relapsed or developed Epstein-Barr virus–induced lymphoma after T cell–depleted BMT were then treated with donor lymphocytes transduced with the herpes simplex virus thymidine kinase (HSV-TK) suicide gene. The transduced lymphocytes survived for up to 12 months, resulting in antitumor activity in five patients. Three patients developed GvHD, which could be effectively controlled by ganciclovir-induced elimination of the transduced cells. These data show that genetic manipulation of donor lymphocytes may increase the efficacy and safety of allo-BMT and expand its application to a larger number of patients.

Allogeneic bone marrow transplantation is the treatment of choice for many hematologic malignancies (*1*, *2*). It is now recognized that the "allogeneic immune advantage," in addition to the effectiveness of high-dose chemoradiotherapy, is responsible for the curative potential of allo-BMT (*1*, *2*). Although the nature of effector cells has not yet been fully elucidated, transplantation of allogeneic bone marrow produces superior results compared to autologous or syngeneic transplants (*3*). However, the therapeutic impact of the allogeneic advantage is limited by the risk of a potentially life-threatening complication, GvHD. Severe GvHD can be circumvented by the removal of T lymphocytes from the graft (*2*). However, T cell depletion increases the incidence of disease relapse, graft rejection, and reactivation of endogenous viral infections (*4*). Thus, the delayed administration of donor lymphocytes has recently been used for treating leukemic relapse after allo-BMT. Patients affected by recurrence of chronic myelogenous leukemia, acute leukemia, lymphoma, and multiple myeloma after BMT could achieve complete remission after the infusion of donor leukocytes, without requiring cytoreductive chemotherapy or radiotherapy (*5*). Other complications related to the severe immunosuppressive status of transplanted patients, such as Epstein-Barr virus–induced B lymphoproliferative disorders (EBV-BLPD) (*6*, *7*) or reactivation of CMV infection (*8*), also benefited. However, severe GvHD represents a frequent and potentially lethal complication of this delayed infusion of donor T cells (*9*). No specific treatment exists for established GvHD. We investigated the genetic manipulation of donor lymphocytes,

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