throughout the experiment, and thymocytes that initially had a high Ca^{2+} concentration ($[Ca^{2+}]_i$ >0.2 μM) remained so, although their $[Ca^{2+}]_i$ oscillated over time. Thus, observed elevations in $[Ca^{2+}]_i$ in thymocytes were not sporadic events of the entire population of thymocytes but rather indicated heterogeneity within the population of thymocytes. Moreover, the oscillations in $[Ca^{2+}]_i$ in thymocytes with large and moderate $[Ca^{2+}]_i$ indicated that these were not terminal elevations of $[Ca^{2+}]_i$ in moribund cells. Indeed, these thymocytes were viable cells by several criteria. (i) They excluded both Trypan blue and propidium iodide. (ii) They could be loaded with and retain indo-1 and fura-2, which requires intracellular esterase activity as well as intact plasma membranes. (iii) Their DNA was intact and not fragmented (*8*).

Finally, we assessed the CD4-CD8 phenotype of αβTg male thymocytes with elevated $[Ca^{2+}]_i$ (Fig. 3). Unlike most thymocytes with a high Ca^{2+} concentration from normal mice (Fig. 3A), thymocytes with a high Ca^{2+} concentration from αβTg male mice (Fig. 3B) were almost entirely $CD4^-CD8^-$, the thymocyte subset that is disproportionately increased in negatively selecting αβTg male mice (Fig. 3B) (*2*). MHC class I–restricted transgenic T cells undergoing negative selection can avoid clonal deletion by reducing their surface expression of CD8 (*7*), which provides an explanation for the increased frequency of $CD4^-CD8^-$ thymocytes in αβTg male mice. We have found that the $CD4^-CD8^-$ phenotype is a specific consequence of antigen encounter and negative selection among developing αβTg male thymocytes (*9*). Thus, the $CD4^-CD8^-$ phenotype of αβTg male thymocytes with elevated $[Ca^{2+}]_i$ is concordant with their having recently undergone and survived thymic negative selection.

Our data indicate that endogenous ligands do stimulate developing thymocytes to mobilize intracellular Ca^{2+} in vivo and that such intrathymic signaling events are evident in thymocytes expressing self-reactive TCR. In vivo elevations in $[Ca^{2+}]_i$ serve as markers of thymocytes whose TCRs have been stimulated by self ligands in the thymus and thus have been subjected to thymic negative selection. We presume that thymocytes that have been negatively selected in vivo to undergo apoptosis also have elevated $[Ca^{2+}]_i$, but most αβTg thymocytes with elevated $[Ca^{2+}]_i$ that are present at the time of measurement are cells that have survived negative selection. Finally, there was no definitive indication in our data that positive selection events in the thymus affected $[Ca^{2+}]_i$, but this issue requires further study.

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12. Thymocytes (1×10^7) were loaded with 1.8 μM indo-1 at 31°C for 30 min in 1 ml of Hanks solution supplemented with 1.0% fetal calf serum, 20 mM Hepes, and 5×10^{-5} M 2-mercaptoethanol (indo medium) (*10*). Cells were washed, incubated at 37°C for 10 min, and then assessed by flow cytometry at 800 cells per second (10^5 cells per histogram) (*10*). In the cell mixture experiment in Fig. 1, the male thymocytes were incubated with 30 μM $DiIC_{18}$ (*5*) for 5 min at 37°C, washed twice, and then mixed with equal numbers of female αβTg thymocytes. The cell mixture was loaded with indo-1, equilibrated at 37°C, and analyzed on a dual laser flow cytometer. Separate analysis of thymocytes from these mice loaded with indo-1 only, and not mixed together, showed that 40% of male and 11% of female αβTg thymocytes had $[Ca^{2+}]_i$ >0.2 μM.
13. The fluorescence digital image processing system used was similar to that of R. Y. Tsien and A. T. Harootunian (*11*). Hardware consisted of an FD5000 image processor (Gould), a Zeiss Axiovert microscope with a 1.3 numerical aperture ×40 objective, and a filter changer (Ludl Electronic Products, Hawthorne, NY) with excitation filters centered at 350 ± 10 nm and 380 ± 10 nm. The image processor and filter changer were interfaced to a Microvax host computer (Digital Equipment Corporation). Images were acquired through a charge-coupled device camera (Cohu, San Diego, CA) and image intensifier (Video Scope). The images were averaged, the background was subtracted, and a shading correction was applied (*11*).
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17. We thank R. Abe and R. Hodes for constructive criticisms, R. Y. Tsien for software for digital image analysis, D. Smoot for flow cytometry, and H. von Boehmer and M. Steinmetz for making their TCR transgenic animals available to the scientific community. C.H.J. was supported by Naval Medical Research and Development Command grant M0095.003-1007.

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Selection of Drug-Resistant Bone Marrow Cells in Vivo After Retroviral Transfer of Human *MDR*1

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Experiments were performed to determine if retroviral-mediated transfer of the human multidrug resistance 1 gene (*MDR*1) into murine bone marrow cells would confer drug resistance to the cells and whether the *MDR*1 gene could be used as a dominant selectable marker in vivo. When mice transplanted with bone marrow cells containing a transferred *MDR*1 gene were treated with the cytotoxic drug taxol, a substantial enrichment for transduced bone marrow cells was observed. This demonstration of positive selection establishes the ability to amplify clones of transduced hematopoietic cells in vivo and suggests possible applications in human therapy.

The failure of certain human tumors to respond to continued chemotherapy is frequently associated with enhanced expression of the multidrug resistance gene, *MDR*1 (*1*). The *MDR*1 gene product, a 170-kD transmembrane protein also known as P-glycoprotein, is an energy-dependent drug efflux pump. This protein extrudes many types of natural products from the cell, including frequently used chemotherapeutic agents, thereby conferring a drug-resistant phenotype to cells expressing this protein.

Bone marrow toxicity of chemotherapeutic drugs is often a dose-limiting side effect in the treatment of cancer patients. Transfer of the *MDR*1 gene to bone marrow cells would therefore be an attractive approach to overcome the myelosuppression associated with such drugs. Earlier studies with a line of transgenic mice containing human *MDR*1 coding sequences showed that these animals were protected from the myelosuppressive effects of a number of natural product chemotherapeutic agents (*2*). A recent study showed that transplan-

tation of bone marrow cells from *MDR*1 transgenic donors protected recipient mice from drug-induced myelosuppression (*3*). The ability to transfer the *MDR*1 gene to bone marrow cells through a retroviral vector (*4*) expands the applicability of this strategy and suggests that *MDR*1 gene transfer may be useful in generating a drug-resistant bone marrow in vivo.

A current limitation of retroviral-mediated transfer of genes into bone marrow cells is that the desired target, the hematopoietic stem cell, is difficult to transduce because it is both rare and often not in cycle. Only 10 to 20% of murine stem cells are transduced with typical protocols (*5*, *6*) although a recent study reports a 70% infection efficiency (*7*). Transduction of pluripotent primate hematopoietic cells is significantly less efficient (*8*). Therefore, another potential use of the *MDR*1 gene would be as a dominant selectable marker in vivo allowing enrichment for the minority of bone marrow cells containing and expressing the recombinant retrovirus.

To test if *MDR*1 gene transfer would allow positive selection of drug-resistant hematopoietic cells in vivo, we used a retroviral vector containing the human *MDR*1 cDNA promoted by the Harvey murine sarcoma virus long terminal repeat (*9*) to generate a high-titer, helper virus–free, ecotropic producer clone (*10*) from the GP+E86 packaging cell line (*11*). Bone marrow cells from donor mice pretreated with 5-fluorouracil to induce stem cell cycling (*12*) were cocultured with producer cells in the presence of murine interleukin-3 and interleukin-6 (IL-3 and IL-6) as described (*5*) and then used to reconstitute recipient W/W^V mice (MDR mice). Mice transplanted with bone marrow transduced with a Moloney murine leukemia vector containing the bacterial neomycin resistance gene were used as controls (Neo^R mice). MDR and Neo^R mice were treated with a single intraperitoneal bolus of taxol and assayed for changes in the number of peripheral blood leukocytes containing the proviral genome and for changes in peripheral blood counts. Taxol is efficiently removed by the *MDR*1 pump, and its nonhematopoietic toxicity is relatively small (*2*, *13*).

Two primer sets were used in the polymerase chain reaction (PCR) to coamplify transferred proviral sequences and endogenous mouse β-globin gene sequences from DNA prepared from mouse blood (*14*). We measured the ratio of provirally derived amplification products to mouse β-globin gene products by densitometry of autoradiograms and compared them to a standard DNA to calculate the average proviral copy number in circulating leukocytes. This number reflects the proportion of circulating cells that contain the proviral genome, assuming one proviral genome per cell. We demonstrated the validity of this quantitative PCR assay by assaying mixtures of DNA obtained from an MDR retroviral producer line (ψ2 MDR) and 3T3 cells (Fig. 1A). A linear increase in MDR signal intensity was seen relative to the β-globin internal control as the proportion of DNA from the producer line was increased from 0 to 100%. Application of this assay to peripheral leukocyte DNA prepared from a set of MDR animals 9 weeks after transplant showed that all nine animals had circulating leukocytes containing proviral sequences but in widely varying amounts (Fig. 1B). Animals M2, M4, and M5 showed a strong MDR signal, indicating that the proportion of leukocytes that contained provirus was high. In contrast, animals M6, M7, and M3 contained few provirally marked leukocytes.

Animals transplanted with cells transduced with the MDR retrovirus expressed significant amounts of viral RNA transcripts in the bone marrow. RNA, prepared from bone marrow of MDR and Neo^R mice 16 weeks after transplant, was reverse transcribed and amplified by PCR (*15*). The cells from the two MDR animals studied showed a greater abundance of *MDR*1

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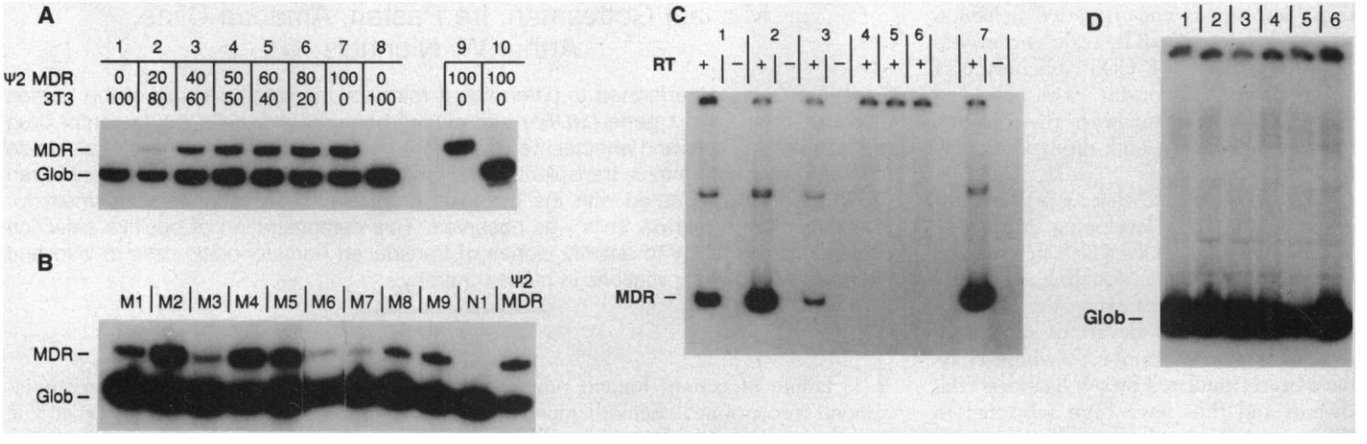


Fig. 1. (**A**) Quantitative PCR assay for *MDR*1 cDNA sequences. The 200 ng of DNA mixtures prepared from the viral producer line ψ2 MDR and NIH 3T3 cells were assayed and are shown in lanes 1 through 10. The percent of DNA from each cell line is shown for each mixture. Lanes 1 through 8 show assays, which used both *MDR*1 primers and β-globin primers. Only *MDR*1 primers were used in lane 9 and only β-globin primers in lane 10. The positions of the *MDR*1- and globin (Glob)-specific bands are indicated on the left. (**B**) Measurement of the proportion of provirally marked leukocytes in nine MDR animals. Peripheral leukocyte DNAs from nine MDR animals (M1 to M9) were assayed 9 weeks after transplant. DNA prepared concurrently from a Neo^R animal (N1) and an MDR producer (ψ2 MDR) cell line are shown as controls. (**C**) RNA analysis for *MDR*1 expression in bone marrow cells from MDR and Neo^R control mice. RNA was PCR amplified as described both with and without a preceding reverse transcription reaction as indicated (± RT). Lanes 1 and 2 show assays from two MDR animals after taxol treatment. Lane 3 shows an assay from an MDR-39 transgenic mouse (*2*). Lanes 4 and 5 are assays of bone marrow RNA from two taxol-treated Neo^R mice and lane 6 is RNA from marrow of a C57Bl mouse. Lane 7 is an assay of RNA from an MDR viral producer cell line. (**D**) β-Globin RNA from bone marrow was amplified as an internal control for mRNA integrity and loading. The lanes are the same as in (C).

mRNA than that observed in bone marrow cells from MDR-39 transgenic mice (2) and only slightly less *MDR*1 mRNA than seen in the viral producer cell line ψ2 MDR (Fig. 1C).

To test if MDR animals were resistant to drug-induced neutropenia, we treated nine MDR animals and nine control animals with taxol 6 months after transplant (*16*). Both groups of animals showed severe myelosuppression 3 days after the first taxol dose, as demonstrated by a large drop in the average neutrophil count (Fig. 2A). Although all MDR mice contained provirally marked cells at 11 weeks after transplant, DNA prepared immediately before the first taxol dose (day 0) showed that in the four mice evaluated, circulating provirally marked cells were no longer detectable (MDR 11 and MDR 12) or were present in low numbers (MDR 10 and MDR 13, Fig. 2B). DNA prepared from these same mice after counts had recovered 13 days later showed the reappearance of circulating leukocytes containing provirus in animals MDR 11 and MDR 12 and a substantial increase in marked cells in animals MDR 10 and MDR 13. When the two groups of animals were rechallenged with taxol on day 17, the average neutrophil count of the MDR group dropped only 7% compared to 42% for the

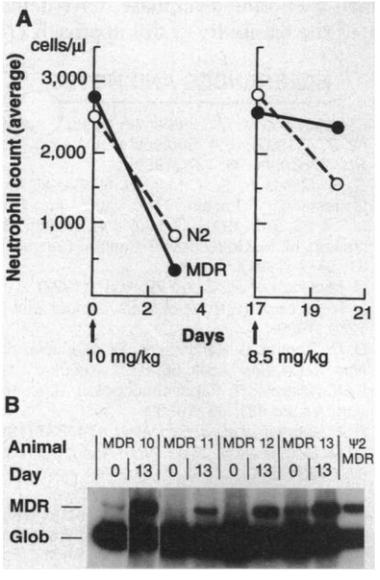


Fig. 2. (**A**) Changes in neutrophil counts in groups of MDR animals and Neo^R animals after treatment with taxol. Nine MDR animals (solid black line) and nine Neo animals (dashed line) were injected intraperitoneally with taxol (10 mg/kg) 24 weeks after transplant (day 0). The average peripheral blood neutrophil counts for both groups were determined before and 3 days after the taxol dose. Both groups were rechallenged with taxol (8.5 mg/kg) 17 days after the first dose and neutrophil counts were again determined. (**B**) Assay for the prevalence of circulating provirally marked leukocytes before and after taxol dosing. Peripheral leukocyte DNA was prepared from four MDR mice both before and 13 days after the first taxol dose and was assayed. DNA from an MDR viral producer line was again used as a positive control. Globin-specific bands are indicated on the left (Glob).

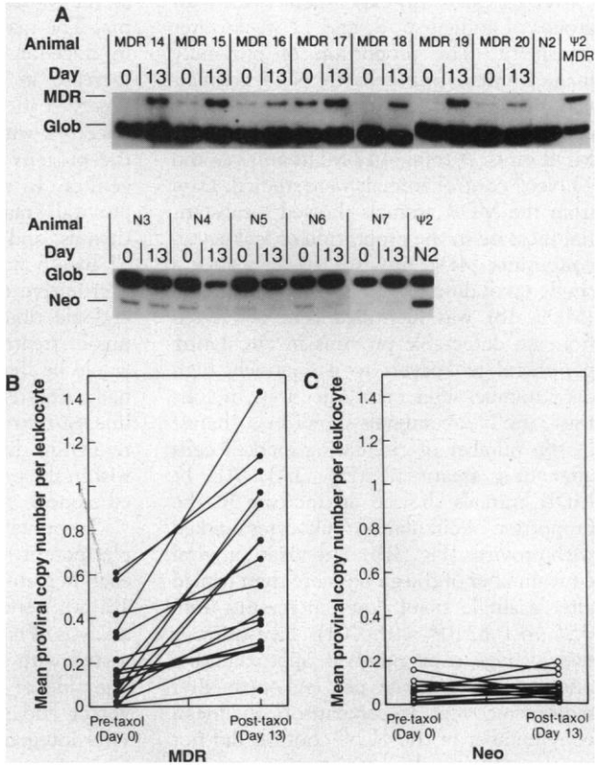
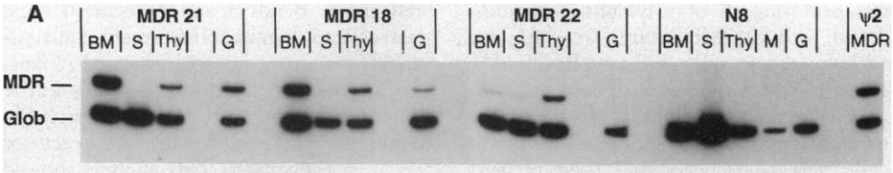


Fig. 3. (**A**) Representative examples of changes in the proportion of marked leukocytes observed with taxol treatment of MDR and Neo^R control animals. The upper panel shows assays of peripheral leukocyte DNA from seven MDR animals (MDR 14 to 20). DNA from a concurrently sampled Neo^R animal (N2) and an MDR producer cell line serves as negative and positive controls, respectively. The lower panel shows representative Neo^R animal assays (N3 to N7). The positions of the globin (Glob) and Neo signals are shown on the left. DNA from the Neo producer cell line, ψ2 N2, was used as a positive control. (**B** and **C**) Complete data set from two separate experiments showing changes in the mean proviral copy number in 17 MDR animals and 13 Neo^R animals treated with taxol. Animals were treated from 6 to 12 weeks after transplant. Autoradiograms in the linear exposure range were scanned with a Bio-Rad densitometer (Richmond, California), and the ratio of MDR (Neo) to β-globin intensity for the animal samples was compared to the ratio obtained from the respective producer cell DNAs. The MDR (Neo) producer cell line contains three (one) proviral copies per genome allowing calculation of the average copy number in experimental samples.



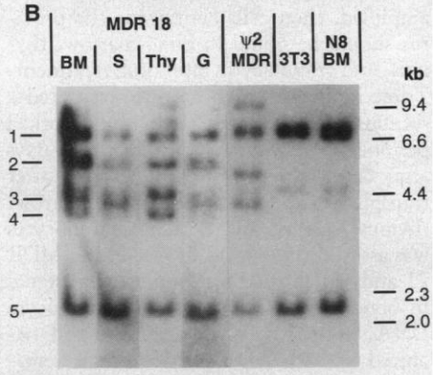


Fig. 4. (**A**) Distribution of MDR provirus in various hematopoietic tissues. DNA was prepared from bone marrow (BM), spleen (S), thymus (Thy), peripheral blood monocytes (M), and granulocytes (G) from three MDR mice (MDR 21, 18, and 22) and one Neo^R mouse (N8) and was assayed for the prevalence of cells marked with MDR provirus. Granulocytes were purified over a Ficoll gradient to a purity greater than 90%. DNA from an MDR producer line was used as a positive control. (**B**) Southern blot analysis for MDR proviral integration sites in hematopoietic tissues from mouse MDR 18. DNA was digested with Hind III, electrophoresed, and blotted onto a filter. The filter was probed with a radiolabeled 864-bp Eco RI–Hind III fragment from the human MDR1 cDNA and washed to a final stringency of 55°C in 0.1× standard saline citrate (SSC). Lanes with DNA from animal MDR 18 are labeled as above. DNA from the viral producer line ψ2 MDR, NIH 3T3 cells, and bone marrow from a concurrently killed Neo^R animal (N8BM) are used as controls. Molecular weight markers are shown on the right, and bands referred to in the text are indicated on the left.

controls (Fig. 2A). Because of variability among individual animals and the small number of animals studied, this difference did not achieve statistical significance.

We repeated this experiment twice with groups of animals 6, 8, and 12 weeks after transplant. The proportion of provirally marked circulating cells in Neo^R animals was measured to test if the enrichment for cells containing provirus was specific for the MDR virus. A total of 17 MDR animals and 13 Neo^R control animals were studied. Data from the MDR animals showed a substantial increase in the proportion of leukocytes containing MDR provirus 13 days after a single taxol dose (Fig. 3A). A third animal (MDR 18) was identified that converted from no detectable provirus in circulating peripheral leukocytes to a relatively high copy number after taxol injection. In contrast, the Neo^R animals showed no change in the number of circulating marked cells after drug treatment (Fig. 3A). All 17 MDR animals showed an increase in the proportion of circulating leukocytes marked with provirus (Fig. 3B). The mean proviral copy number of the group more than tripled after a single taxol dose, increasing from 0.20 to 0.62 ($P < 0.0001$). Five animals had average copy numbers approximating one proviral genome per leukocyte after marrow recovery. In comparison, the mean copy number of the Neo^R controls did not change with taxol administration, remaining low at an average of 0.07 copies per cell (Fig. 3C), equivalent to 7% of cells containing a proviral genome.

We studied the tissue distribution of provirally marked cells to determine the hematopoietic lineages in which selection was occurring. If only lymphocytes transduced with the MDR virus were selected with taxol, provirally marked cells would be expected in the thymus but not in the bone marrow or among peripheral blood granulocytes. Conversely, if pluripotent marrow cells were transduced, and their progeny amplified, then cells containing the provirus should be found in bone marrow, thymus, and granulocytes after drug treatment. Three animals, which had demonstrated a substantial increase in retrovirally marked peripheral leukocytes, were killed 21 days after taxol administration (15 weeks after transplant), and DNA from bone marrow, thymus, spleen, and purified granulocytes was assayed (Fig. 4A). Two animals (MDR 21 and 18) showed a strong proviral signal in bone marrow, thymus, and granulocyte DNA, suggesting that selection had occurred at the level of multipotential hematopoietic stem cells. One animal (MDR 22) showed a strong MDR signal only in thymus DNA, weak signals in the bone marrow and spleen DNA, and no detectable provirus in the granulocyte DNA.

This animal to animal variability in the transduced cell populations may explain the differing degree of enrichment for marked cells seen among individual animals as well as the variable protection from neutropenia. The majority of circulating leukocytes in mice are lymphocytes, and the large increase in provirally marked leukocytes observed in MDR 22 is best explained by selection within the lymphoid lineage for the progeny of a lymphoid-restricted progenitor. In contrast, the large numbers of provirally marked cells in the bone marrow, thymus, and granulocytes of animals MDR 21 and 18 most likely reflect transduction of a primitive multipotent bone marrow cell and selection of that cell or its progeny with taxol treatment. Another explanation would be that separate transduction events had occurred involving several different lineage-restricted progenitors. This would result in different viral integration sites within the progeny of these lineage-restricted clones.

To prove that the progeny of individual pluripotent stem cells were represented in each hematopoietic lineage in mouse MDR 18, we performed Southern (DNA) blot analysis (Fig. 4B). DNA samples were digested with Hind III, which cuts only on one side of the *MDR*1 probe within the vector and gives a unique band for each viral integrant. Bands 1 and 5 are seen in DNA from 3T3 cells and a Neo^R animal and represent hybridization of the probe to endogenous mouse genomic MDR sequences. Band 2 occurs in DNA of all hematopoietic tissues from MDR 18 and therefore is likely to be derived from the progeny of a single retrovirally marked stem cell clone. Bands 3 and 4 occur in bone marrow and thymus and represent contributions from other lineage-restricted clones. An intermediate band was seen only in the spleen and granulocyte DNA and probably represents marking of a myeloid precursor clone in the spleen. This analysis showed transduction of pluripotent stem cells as well as more differentiated lineage-restricted progenitors with progeny from both contributing to myeloid and lymphoid lineages after in vivo selection.

These results demonstrate that the *MDR*1 gene can function as a dominant selectable marker in vivo, allowing a minority of genetically altered bone marrow cells to be positively selected by cytotoxic drug administration. Similarly designed experiments with a retrovirally transferred dihydrofolate reductase gene resulted in protection of murine recipients from methotrexate-induced cytopenias. However, enrichment for transduced cells could not be demonstrated (*17*). Differences in drug regimens and vector design may, in part, explain the different results.

Although it is unproven at which level in hematopoiesis that selection was occurring, our data suggest that positive selection of *MDR*1-transduced cells may occur at the stem cell level in some animals. The initial absence but subsequent appearance of circulating provirally marked leukocytes in three MDR animals is best explained by selection of an *MDR*1-expressing stem cell clone that was quiescent before taxol administration (*18*). Our failure to observe this phenomenon in control animals supports this interpretation.

Retroviral-mediated *MDR*1 gene transfer may provide a means to generate a drug-resistant bone marrow in human cancer patients undergoing bone marrow transplantation. Another potential clinical application of this strategy would be the use of in vivo selection to overcome the anticipated inefficiency of gene transfer with nonselectable genes such as β-globin. Experiments describing a retroviral vector, which expressed a bifunctional chimeric protein formed from the *MDR*1 transporter and human adenosine deaminase, have demonstrated the feasibility of this approach (*19*).

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10. Initially, DNA from plasmid pHaMDR1/A was introduced by calcium phosphate cotransfection into an amphotrophic packing line, PA317. Forty-eight hours later, we used medium conditioned by these cells to infect GP+E86 cells and obtained a high titer producer clone by colchicine selection.
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14. Blood (150 μl) was obtained from the retro-orbital venous plexus of mice. Red cells were lysed, and DNA was prepared from pelleted leukocytes. A 401–base pair (bp) sequence specific for the mouse beta-major gene was amplified with the following primers: left-hand primer, 5′-GAAGTTGGGTGCTTGGAGAC-3′, and right-hand primer, 5′-GAGACTGCTCCCTAGAATCG-3′. We amplified a 494-bp sequence specific for the human *MDR*1 gene with the following primers: left-hand primer, 5′-GCCCACATCATCATGATC-3′, and right-hand primer, 5′-GTCTCCTACTTTAGTGCT-3′. A 335-bp sequence specific for the Neo-containing provirus was amplified with the, following primers: left-hand primer, 5′-GGACCTTGCACAGATAGCGT-3′, and right-hand primer 5′-CT-

GTTCCTGACCTTGATGTG-3'. The final concentration of each primer in the reaction mixture was 1.0 μM. The 200 ng of DNA served as template under reaction conditions recommended by the manufacturer (United States Biochemical) except that two sets of primers were used and 0.4 μl of (α-^{32}P)dCTP 800 Ci/mmol were added to each reaction. Amplification was for 20 cycles with an annealing temperature of 58°C for the *MDR*1 primers and 55°C for the Neo primers. We separated amplified products on a 5% nondenaturing polyacrylamide gel and visualized them by autoradiography of the dried gel.

15. Mice were killed by cervical dislocation, and bone marrow cells were flushed from long bones. Total cellular RNA (cRNA) was prepared by extraction in guanidine isothiocyanate and treated with DNase I. The 2 μg of RNA were reverse transcribed and PCR amplified as previously described [B. P. Sorrentino *et al.*, *Nucleic Acids Res.* **18**, 2721 (1990)]. Using the above described β-globin or MDR primers in separate reactions, we amplified for 30 cycles with an annealing temperature of 58°C.
16. Taxol was obtained from the Developmental Therapeutics Branch of the National Cancer Institute and was dissolved in 30% dimethyl sulfoxide (DMSO), 30% ethanol, and 40% sterile water immediately before use. This carrier had no effect on peripheral blood counts. White blood counts were measured both by manual counting and automated Coulter analysis of each sample. White blood cell differentials on Wright stained blood films were performed on 100 cells per sample and used to calculate the absolute neutrophil counts.
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Infection of *Macaca nemestrina* by Human Immunodeficiency Virus Type–1

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After observations that *Macaca nemestrina* were exceptionally susceptible to simian immunodeficiency virus and human immunodeficiency virus type–2 (HIV-2), studies of HIV-1 replication were initiated. Several strains of HIV-1, including a recent patient isolate, replicated in vitro in peripheral blood mononuclear cells (PBMCs) and in CD4-positive *M. nemestrina* lymphocytes in a CD4-dependent fashion. Eight animals were subsequently inoculated with either cell-associated or cell-free suspensions of HIV-1. All animals had HIV-1 isolated by cocultivation, had HIV-1 DNA in their PBMCs as shown by polymerase chain reaction, and experienced sustained seroconversion to a broad spectrum of HIV-1 proteins. *Macaca nemestrina* is an animal model of HIV-1 infections that provides opportunities for evaluating the pathogenesis of acute HIV-1 replication and candidate vaccines and therapies.

A relevant animal model (*1*) for human lentivirus infection (*2*, *3*) is needed for the evaluation of the effectiveness of vaccines and drugs for HIV-1 infection. Asian macaques infected by the related simian immunodeficiency virus (SIV) (*4*, *5*) or HIV-2 (*6*) have been used to study the acquired immunodeficiency syndrome (AIDS). Although this animal model has contributed to our understanding of viral pathogenesis, SIV and HIV-2 differ from HIV-1 in their sequence homology and antigenic structure (*7*). These viral sequence differences are especially pertinent to HIV-1 vaccine development, in which an animal model that would allow testing of candidate HIV-1 vaccines would be particularly useful. Among nonhuman primates, only the endangered chimpanzee and gibbon ape are susceptible to HIV-1 infection (*8*–*10*). We evaluated *Macaca nemestrina* (pigtail macaques) as a model for HIV-1 infection on the basis of clinical and virological observations that suggested this species was more severely affected by SIV and HIV-2 infection than *Macaca mulatta* or *Macaca fascicularis* (*11*).

Four HIV-1 strains were tested for their ability to replicate in macaque PBMCs in vitro: HIV-1_{LAI}, obtained from the Pasteur Institute (*2*, *12*), HIV-1_{NL4-3} [a molecular clone (*13*)], HIV-1_{JR-CSF} [the molecular clone of an isolate originally cultured from the cerebrospinal fluid of a patient with AIDS dementia (*14*)], and HIV-1_{RO} (a recent patient isolate). Stocks of each isolate were prepared and titrated on human PBMCs (*15*). We initially assessed the ability of HIV-1_{LAI} to infect the PBMCs from three species of macaques and humans. In addition, we infected the macaque PBMCs with SIV_{mne} (*16*) and heat-inactivated HIV-1 and SIV. The HIV-1_{LAI} replicated efficiently in both *M. nemestrina* and human PBMCs, whereas no detectable replication occurred in *Macaca mulatta* or *Macaca fascicularis* PBMCs (Table 1). The SIV_{mne} replicated in PBMCs from all three macaque species, but viral antigens were not detected in the cultures infected with the heat-inactivated viruses. Furthermore, HIV-1_{NL4-3}, HIV-1_{JR-CSF}, and HIV-1_{RO} replicated in the *M. nemestrina* cells as assayed by HIV-1 antigen production (Table 1).

We further characterized the in vitro infection by determining whether *M. nemestrina* CD4-positive cells were the target of HIV-1 infection. Unfractionated PBMCs and purified CD4 cells, isolated from *M. nemestrina* and humans, were infected with HIV-1_{LAI}. The HIV-1 replicated with similar efficiency and kinetics in the macaque and human CD4-positive cells. The HIV-1 antigen in culture supernatants was detected sooner and at higher titers in the CD4-enriched cell populations (Table 1) as compared to that in the unfractionated cells, which is consistent with the known affinity of HIV-1 for CD4-positive cells in humans (*17*). We abrogated the infection by first treating the virus with human recombinant soluble CD4. Syncytial formation was observed microscopically in these cultures, although infrequently when compared to that in human CD4 cells infected by HIV-1 (*18*).

To provide further evidence of HIV-1 replication in *M. nemestrina* PBMCs, we examined de novo viral protein synthesis by radioimmunoprecipitation analysis (*19*). HIV-1–specific protein synthesis could be detected by day 5 in the macaque PBMCs (Fig. 1). The migration of the proteins from HIV-1–infected cells was distinct from the migration of proteins from SIV- and HIV-2–infected cells; this was particularly true for the envelope glycoprotein and the major gag proteins. Further, monoclonal antibodies specific for HIV-1 *env* and *gag* gene products (*20*) recognized viral proteins synthesized in cells infected by HIV-1, but not in cells infected by SIV_{mne} or HIV-2 (*18*).

We evaluated whether the in vitro HIV-1 infection in macaque PBMCs resulted in the formation of complete virus particles. Seven days after infection, electron microscopy revealed that large amounts of mature virions with the characteristic lentivirus morphology were associated with cells or cellular processes. Occasional budding forms associated with the plasma membrane of cultured

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