The human and African green monkey TRIM5α genes encode Ref1 and Lv1 retroviral restriction factor activities

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Edited by John M. Coffin, Tufts University School of Medicine, Boston, MA, and approved June 7, 2004 (received for review April 7, 2004)

The rhesus macaque tripartite motif containing protein TRIM5α specifically restricts HIV-1 infection at an early post-entry step before reverse transcription [Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P. & Sodroski, J. (2004) *Nature* 427, 848–853]. Here, we show that the human and African green monkey (AGM) TRIM5α genes encode Ref1 and Lv1 antiretroviral activities, respectively. Expression of TRIM5α in permissive cat cells renders them resistant to restriction-sensitive murine leukemia virus but not closely related insensitive virus. Disruption of TRIM5α expression in human and AGM cells with small interfering RNA rescues infectivity of restricted virus without affecting unrestricted virus. We also demonstrate that the activity of the murine restriction factor Fv1 depends on TRIM5α expression when Fv1 is expressed in human cells. Furthermore, a drug that modifies the behavior of the related promyelocytic leukemia protein PML specifically rescues infection by viruses restricted by human TRIM5α. Alignment of the TRIM5α proteins from rhesus macaque and AGM indicates an 18-aa insertion. We speculate that this insertion may contribute to the broader specificity of the AGM TRIM5α restriction as compared with the human and rhesus macaque proteins.

Retroviruses are obligate parasites that depend on a number of host cell functions to complete their lifecycle. An approach to identify the normal cellular processes required by retroviruses has been to study instances of species-specific retroviral replication. Primate lentiviruses tend to have narrow host ranges, making them ideal for studying factors controlling species specificity. The study of human cell lines differentially permissive for HIV-1 led to the identification of the CEM15/Apobec3G protein able to block HIV-1 infection (1). This protective host factor induces catastrophic deamination of the viral DNA as it is synthesized (2). The sole function of the HIV-1 *vif* gene seems to be to deactivate the host CEM15/Apobec3G protein, thereby allowing infection (3–8). Certain primate cell lines have been found to be differentially permissive for primate lentiviral vectors, even when specific receptor requirements are obviated by pseudotyping with the broadly tropic envelope protein VSV-G from vesicular stomatitis virus (9). Recently, it has been shown that such non-permissivity is often due to saturable, dominant-acting restriction factors that are able to effectively block infection after susceptible virus enters the host cell (10–12).

These restriction factors are exemplified by the murine factor Fv1, active against murine leukemia viruses (MLV) (13). There are two principle alleles of *Fv1*, namely *Fv1 N*, found in NIH mice, and *Fv1 B*, found in BALB/c mice. The two alleles allow the division of MLV isolates into N-tropic viruses, able to infect NIH cells but not BALB/c cells, and B-tropic viruses able to infect BALB/c cells but not NIH cells. These mice, cell lines derived from these mice, and cell lines exogenously expressing the Fv1 proteins are able to specifically block MLV infection after reverse transcription but before integration of the provirus (14–17). The viral determinant for Fv1 restriction is at position 110 in the capsid (CA) protein. A glutamate specifies B-tropism and an arginine specifies N-tropism (18). The common lab strain of MLV, Moloney MLV (19) is NB-tropic, able to infect both Fv1 N- and B-positive cells. The changes rendering a virus NB-tropic are complex and have not been described. The Fv1 gene has been cloned and seems to be a retroviral gag, unrelated to MLV, presumably a remnant of an ancient proviral sequence (16). The mechanism of Fv1 restriction remains unclear (reviewed in refs. 20 and 21). Rodent cells are also able to restrict lentiviruses through an Fv1-independent mechanism (22).

Remarkably, cells from a range of mammals, including humans, are able to restrict N-tropic MLV but not B-tropic MLV at an early step, post-entry, before reverse transcription, and with the same specificity for arginine at CA110 as Fv1 B (23). This observation suggested a human Fv1 allele, but Southern blot of human DNA failed to detect closely related sequences (16, 17). The human factor was named Ref1, and further characterization revealed the ability of Ref1 to block infection by the horse lentivirus equine infectious anemia virus (EIAV) (24, 25). Analysis of restriction in simian cells demonstrated different restriction sensitivities in different species. The simian restriction factor was dubbed Lv1. Lv1 from African green monkey (AGM) cells was shown to block a broad range of retroviruses including the gamma retrovirus MLV-N as well as lentiviruses HIV-1, HIV-2, simian immunodeficiency virus macaque (SIVmac), and EIAV (10–12, 23). All Lv1-mediated blocks are early post-entry, before reverse transcription. Experiments where restriction to one virus is saturated by exposure to a second unrelated virus have shown that, in each species, there is a common saturable component that, when saturated, makes cells permissive to all restricted viruses (25). How viruses such as HIV-1 are able to bypass restriction by Ref1 is of interest. At least in the case of HIV-1, it seems that insensitivity to Ref1 is mediated through an interaction with the cellular protein cyclophilin A (CypA) by means of an exposed loop on the CA (26). The simplest model for this is that CypA acts by altering the availability of the CA for Ref1 binding. Intriguingly, preventing CypA-CA interactions during infection of simian cells stimulates infection, suggesting that maximal restriction requires a CypA-CA interaction to occur (26).

Recently, a host factor blocking HIV-1 infection in cells from rhesus macaque has been identified as the tripartite motif containing protein TRIM5α (27). Here, we identify the human

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: AGM, African green monkey; MLV, murine leukemia virus; EIAV, equine infectious anemia virus; RFP, red fluorescent protein; FACS, fluorescence-activated cell sorter; CA, capsid; MDTF, *Mus dunni* tail fibroblasts; siRNA, small interfering RNA; PML, promyelocytic leukemia.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AY593973).

See Commentary on page 10496.

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and AGM TRIM5α proteins as having activities identical to Ref1 in humans and Lv1 in AGMs, respectively.

Materials and Methods

Cell Lines, Viral Vectors, and Infections. HeLa, TE671, 293T, COS7 *Mus dunni* tail fibroblasts (MDTF) and SC1 cells were obtained from the American Type Culture Collection (ATCC) and have been described (10, 23). MDTF cells are Fv1 null, and SC1 is a feral mouse embryo cell line. Neither restricts MLV-N, MLV-B, or MLV-NB (28). TEN cells (TE671 expressing Fv1N) have been described (17). The feline kidney cell line CRFK was a gift from Yasuhiro Ikeda, University College London. All cells were grown in DMEM, 10% FCS, and 1 unit/ml penicillin and streptomycin.

Vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped MLV, EIAV, and HIV-1 retroviral vectors encoding GFP were prepared by transfection of 293T cells with FuGENE 6 (Roche) as described (10, 23, 25). Infection assays were performed in 24-well plates containing 2.5×10^4 target cells per well. Infected cells were enumerated by fluorescence-activated cell sorter (FACS, Becton Dickinson) 48 h after infection as described (10, 23). MLV titers were equalized on permissive SC1 cells and where relevant titers are described as SC1 infectious units.

Viral reverse transcriptase activity was measured by using RT ELISA (Cavidi Tech, Uppsala). TaqMan PCR to measure viral DNA synthesis 6 h after infection was performed by using primer/probes sequences specific to GFP as described (10).

Arsenic trioxide (As_2O_3) was purchased from Sigma and reconstituted in PBS as described (29). Cells were treated with As_2O_3 at the time of infection.

Expression of TRIM5α. The TRIM5α gene was amplified by PCR from human IMAGE clone no. 6160154 (MRC Gene Service, Cambridge, U.K.) by using primers forward 5′-CAGACGAATTCCACCATGGCTTCTGGAATCCTGGTTAATG-3′ and reverse 5′-ATCGTTCGAATCAAGAGCTTGGTGAGCACAGAG-3′. It was cloned into the MLV vector CXCR [a derivative of CNCG (23)] into the Bcl1 Csp45I sites such that it was expressed under the control of the MLV LTR in infected cells. This vector also encodes red fluorescent protein (RFP, Clontech) under control of the cytomegalovirus (CMV) immediate early promoter in infected cells. TRIM5α, RFP-encoding vector was prepared by 293T transfection by using NB-tropic MLV core and a vesicular stomatitis virus glycoprotein (VSV-G) envelope as described (10). CRFK or MDTF cells were transduced with vector encoding TRIM5α and RFP. Single-cell clones were isolated by limiting dilution, and positive clones were identified by fluorescence microscopy. Positive clones were then infected with MLV-N or -B encoding GFP, and, 48 h after that, cells were assayed for green fluorescence by FACS as described (30). AGM TRIM5α was PCR amplified from a cDNA library derived from AGM *Cercopithicus* sp. CV1 cells and cloned into CXCR as above. It was tested for activity as for human TRIM5α above. Human TRIM5α sequence was verified by DNA sequencing. AGM TRIM5α was determined by sequencing three independent clones. DNA analysis was performed with DNA COWBOY (Blue Tractor Software, Malltraeth, U.K.).

Disruption of TRIM5α Expression by Transfection with Small Interfering RNA (siRNA). Disruption of TRIM5α expression by siRNA was performed by transfecting cells with siRNA oligos (Qiagen, Valencia, CA) by using Oligofectamine (Invitrogen) as described (31). siRNA oligo sequences to TRIM5α have been described (27). We have used sequences 1 and 3. Forty-eight hours after transfection, cells were infected with GFP-encoding retroviral vectors. Green fluorescing, infected cells were enumerated by FACS 48 h after infection. siRNA on AGM COS7 cells was performed as above by using siRNA oligo no. 1 (27). Control siRNA transfections were performed with a negative control siRNA against the μ2 subunit of AP2 (31).

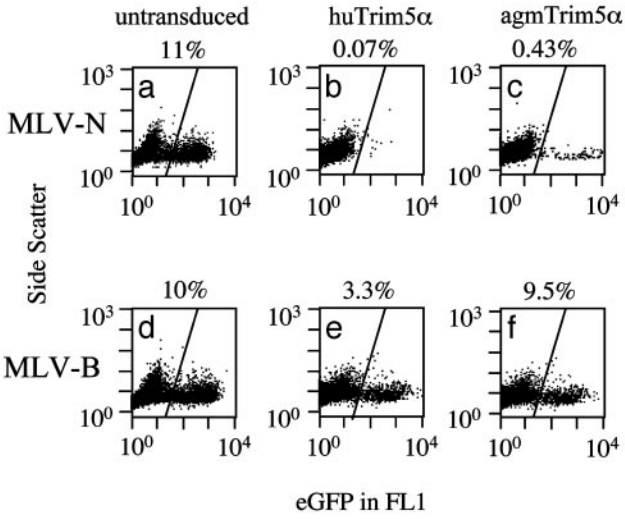

Fig. 1. Expression of human or AGM TRIM5α in cat cells enables them to restrict MLV-N but not MLV-B. Cat CRFK cells were transduced by a retroviral vector encoding TRIM5α from human (*b* and *e*) or AGM (*c* and *f*), and single-cell clones were isolated. Untransduced CRFK cells and TRIM5α-positive clones were infected with equivalent doses of MLV-N (*a–c*) or MLV-B (*d–f*) encoding GFP. The multiplicity of infection was 0.1. Forty-eight hours later, the cells were analyzed for green fluorescence by FACS. Side scatter is shown on the *y* axis, and GFP or MLV infectivity is shown on the *x* axis. Percentages given indicate the proportion of GFP-positive cells (MLV infected). Data shown are representative of experiments performed on 12 independent TRIM5α-positive CRFK clones. eGFP, enhanced GPP.

Results

Human and AGM TRIM5α Genes Block Infection by MLV-N When Expressed in Cat Cells. The recent demonstration that the TRIM5α protein from rhesus macaques is able to block HIV-1 infection has led us to examine whether the human TRIM5α gene can block MLV-N infection. We cloned the human TRIM5α-encoding cDNA into a retroviral vector also encoding RFP. TRIM5α, RFP-encoding MLV virus was prepared and used to transduce feline CRFK cells. These cells were chosen because they have been shown to be very permissive to retroviral infection and equally permissive to MLV-N and MLV-B (ref. 32 and data not shown). Single-cell clones expressing RFP were then infected with N- or B-tropic vectors encoding GFP. Forty-eight hours later, green-infected cells were enumerated by FACS (Fig. 1). These data show that the cells expressing human TRIM5α are approximately >2 orders of magnitude less permissive for MLV-N than untransduced cat cells. Furthermore these cells are ≈3-fold less permissive for MLV-B than untransduced cells. We performed a similar analysis on cells transduced with a vector encoding AGM TRIM5α and RFP. In this case, the AGM TRIM5α-positive cells were able to block MLV-N by ≈1 order of magnitude, but MLV-B infectivity was unaffected. Similar results were obtained from experiments performed in Fv1 null, MDTF cells (Fig. 5 and data not shown)

Disruption of TRIM5α Expression by siRNA Specifically Rescues Infectivity of Restricted Virus in Human and AGM Cells. To confirm the identity of human and AGM TRIM5α as Ref1/Lv1, respectively, siRNA was used to down-regulate TRIM5α expression in HeLa and COS7 cells, as described (27). We then measured permis-

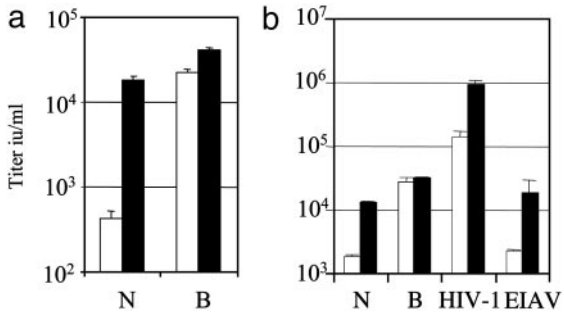

Fig. 2. Disruption of TRIM5α expression by siRNA in human or AGM cells increases permissivity for restricted virus but not unrestricted virus. Human HeLa cells (*a*) or AGM COS7 cells (*b*) were transfected with siRNA to TRIM5α (filled bars) or left untransfected as control (open bars) and infectious titer of restricted and nonrestricted viruses were determined by infection followed by assay of GFP by FACS 48 h later. Infections were performed such that between 0.5% and 10% target cells were infected to ensure linearity. Errors are SEM. Results are representative of two independent experiments.

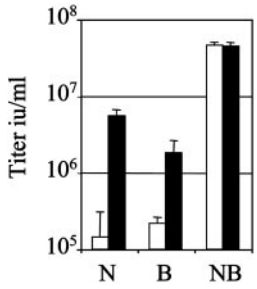

Fig. 4. Disruption of TRIM5α expression by siRNA in human cells expressing Fv1 N rescues infectivity of both TRIM5α and Fv1 restricted infection but not unrestricted infection. Human TEN cells expressing Fv1 N were transfected with siRNA to TRIM5α (filled bars) or left untransfected as control (open bars), and infectious titers of restricted and nonrestricted virus were determined by infection followed by assay of GFP by FACS 48 h later. Infections were performed such that between 0.5% and 10% target cells were infected to ensure linearity. Errors are SEM. Results are representative of two independent experiments.

sivity to restricted and unrestricted virus (Fig. 2). Untransfected HeLa cells or HeLa cells transfected with an irrelevant oligo (data not shown) were between 1 and 2 logs less permissive for MLV-N than MLV-B vector as described (23). However, after transfection of siRNA against human TRIM5α, the titer of the MLV-N was increased to that of MLV-B. Similarly, down-regulation of TRIM5α expression in AGM COS7 cells rescued permissivity to restricted virus without affecting unrestricted virus. AGM cells restrict MLV-N, HIV-1, EIAV, simian immunodeficiency virus macaque (SIVmac), and HIV-2 (23, 25). After siRNA oligo 1 transfection (27) the titer of MLV-N, HIV-1, and EIAV was increased by 10- to 20-fold, but the titer of unrestricted MLV-B was unaffected.

Disruption of TRIM5α Expression by siRNA Rescues MLV-N DNA Synthesis in Human Cells. The block to infection of MLV-N in human cells is early, before reverse transcription (23). We would therefore expect that rescue of MLV-N infection by siRNA to TRIM5α would also rescue MLV-N's ability to reverse transcribe. We therefore compared viral DNA synthesis in human HeLa cells after infection by MLV-N or B after transfection with TRIM5α siRNA. Untransfected cells were infected as a control. Viral DNA was measured 6 h postinfection by TaqMan quantitative PCR as described (10) (Fig. 3). Untransfected cells support 1–2 logs less reverse transcription after infection by MLV-N than after infection with MLV-B. After disruption of TRIM5α expression by siRNA treatment, MLV-N was able to synthesize 10 times more viral DNA, indicating that blocking TRIM5α expression rescues the ability of MLV-N to reverse transcribe and hence increases infectivity.

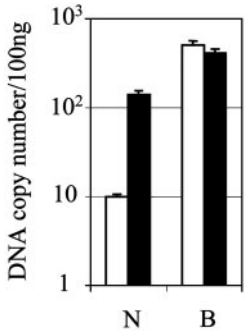

Fig. 3. Disruption of TRIM5α expression by siRNA in human cells increases MLV-N but not MLV-B viral DNA synthesis. Human HeLa cells were transfected with siRNA to TRIM5α (filled bars) or left untransfected as control (open bars) and infected with equivalent doses of MLV-N and MLV-B encoding GFP. Six hours after infection, total DNA was purified and subjected to TaqMan quantitative PCR as described in *Materials and Methods*. DNA copy number per 100 ng of total DNA is plotted. Errors are SEM. Results are representative of two independent experiments.

Down-Regulation of TRIM5α Inactivates Fv1 Restriction in Human Cells. It is intriguing that cells from a range of mammals are able to restrict MLV-N but not MLV-B with an exquisite specificity for arginine at amino acid 110 in the MLV CA (17, 23). It seemed likely that the explanation might lie in relatedness between the restriction factors themselves. Murine Fv1 is related to retroviral gag so it seemed possible that Ref1 and $Lv1_{AGM}$ might also be gag-like. The identification of TRIM5α as encoding Ref1/$Lv1_{AGM}$ shows that they are not, and we must seek an alternative explanation. A possibility is that Fv1 depends on TRIM5α and acts to modify endogenous TRIM5α specificity. To test this hypothesis, we used a human cell line that expresses murine Fv1 N (TEN) (10). This TE671 cell line derivative is able to restrict MLV-N, through endogenous TRIM5α, and MLV-B, through exogenously expressed Fv1 N. We down-regulated TRIM5α expression by siRNA in TEN cells and compared permissivity to TRIM5α-restricted MLV-N, Fv1-restricted MLV-B, and unrestricted MLV-NB (Fig. 4). Both negative controls, untransfected cells, and cells transfected with irrelevant siRNA (data not shown) are able to restrict both MLV-N and MLV-B but not MLV-NB. However, cells transfected with siRNA to TRIM5α become at least 10-fold more permissive to MLV-N in concordance with data in Fig. 2. Remarkably, permissivity to MLV-B is also rescued by down-regulation of TRIM5α expression (Fig. 4). The rescue of MLV-B infectivity is slightly lower than the rescue of MLV-N, but it is clear that the siRNA-transfected cells are significantly more permissive for both restricted viruses, whereas the titer of unrestricted MLV-NB remains unchanged. These data clearly show a functional dependence of Fv1 activity on TRIM5α expression in human cells.

Arsenic Trioxide Treatment Rescues Infection of Human Cells by Both EIAV and MLV-N. The identification of Ref1 as TRIM5α and as a tripartite motif containing protein suggests an explanation for the intriguing ability of arsenic trioxide to overcome the Ref1-mediated block to MLV-N (29). Arsenic trioxide has been used with great success to treat promyelocytic leukemia (33). This leukemia is caused by an oncogenic fusion protein

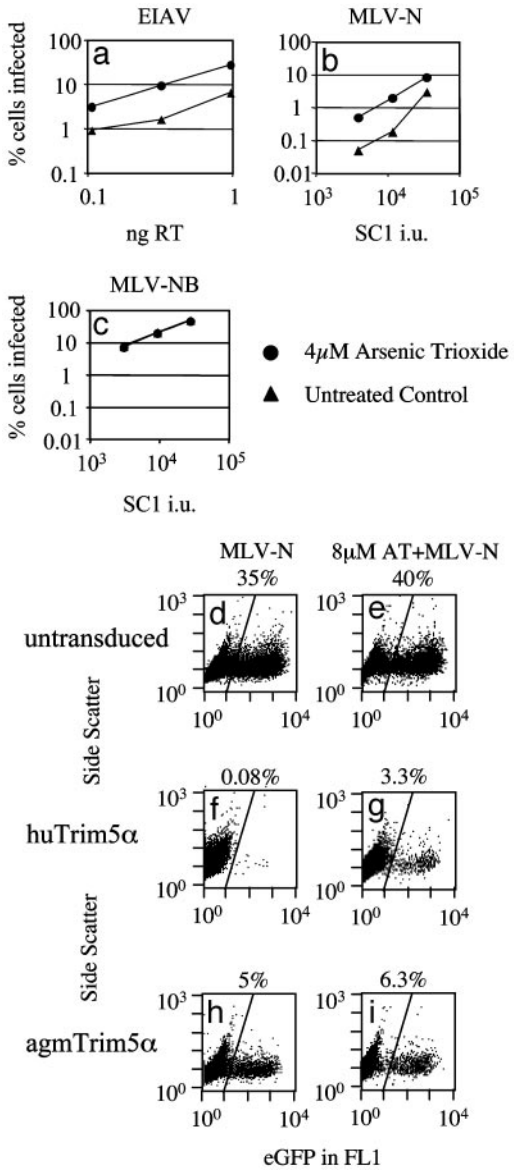

Fig. 5. Arsenic trioxide specifically rescues viral infectivity from restriction by human but not AGM TRIM5α. Human TE671 cells were infected with titrations of restricted EIAV (*a*), restricted MLV-N (*b*), and unrestricted MLV-NB (*c*). Infections were performed in the presence ● or absence ▲ of 4 μM arsenic trioxide (AT). Viral doses are measured in nanograms of reverse transcriptase (EIAV) or SC1 infectious units (MLV). Untransduced (*d* and *e*), human TRIM5α-expressing (*f* and *g*), and AGM TRIM5α-expressing (*h* and *i*) murine MDTF cells were infected with MLV-N GFP at a multiplicity of infection of 0.4 in the presence and absence of 8 μM AT. Forty-eight hours later, infected cells were enumerated by FACS. In *d–i*, percentages given indicate the proportion of GFP-positive cells (MLV-infected). Data shown are representative of three independent experiments.

encoded by a chromosomal translocation joining the promyelocytic leukemia protein (PML) to the retinoic acid receptor protein. How arsenic trioxide mediates this effect is unclear, but its chemotherapeutic abilities are clearly related to its effects on PML localization and degradation (34). The function of the wild-type PML protein remains a mystery and has long intrigued cell biologists due to its location in discrete nuclear bodies. Both TRIM5α and PML are members of the tripartite motif containing family of proteins. It seems likely therefore that the rescue of MLV-N infection by As_2O_3 is due to deactivation and/or degradation of TRIM5α. If this is the case, then we would expect As_2O_3 to rescue Ref1-restricted EIAV infection in the same way it rescues MLV-N infection. In fact, 4 μM As_2O_3 rescues infectivity of EIAV-GFP by 5- to 10-fold (Fig. 5*a*). As described, 4 μM As_2O_3 was able to rescue Ref1-restricted MLV-N and convert the kinetics of infection from multiple hit (slope >1), indicative of restriction, to 1 hit (slope ≈1), indicative of permissive infection (Fig. 5*b*). As_2O_3 treatment did not affect infectivity by unrestricted MLV-NB (Fig. 5*c*).

Intriguingly the titer of restricted MLV-N is unaffected by arsenic treatment in AGM cells, (ref. 29 and data not shown). We therefore compared the effect of As_2O_3 on the titer of MLV-N in MDTF cells expressing human or AGM TRIM5α genes or untransduced cells as a control (Fig. 5). The titer of MLV-N was unaffected by arsenic treatment in TRIM5α negative cells (Fig. 5 *d* and *e*). Expression of human TRIM5α (Fig. 5*f*) or AGM TRIM5α (Fig. 5*h*) was able to block MLV-N infection by 3 or 1 orders of magnitude, respectively. In concordance with the effect of As_2O_3 on human and AGM cell lines, the human TRIM5α restriction of MLV-N was rescued by 2 orders of magnitude by As_2O_3 (compare Fig. 5 *f* to *g*), but AGM TRIM5α-restricted infection was unaffected by As_2O_3 treatment (compare Fig. 5 *h* to *i*) in MDTF cells. The titer of MLV-B remained unrestricted and therefore unaffected by As_2O_3 in all cases (data not shown). These data indicate that the human and AGM TRIM5α proteins themselves are responsible for the differential arsenic sensitivities rather than differences between human and AGM cells unrelated to restriction. The MDTF cells are able to tolerate 8 μM As_2O_3 without obvious toxicity. Similar data were obtained by using feline CRFK clones expressing human and AGM TRIM5α (data not shown).

Discussion

Recent investigation of host factors controlling species-specific retroviral infection has emphasized the role of genetically dominant host factors able to protect cells from retrovirus infection. A significant finding has been the identification of rhesus macaque TRIM5α as the factor blocking HIV-1 infection of cells from rhesus macaque (27). Although there are likely to be further blocks to HIV-1 replication in rhesus macaque monkeys, such as rhesus Apobec3G, it is clear that TRIM5α is a major determinant of permissivity in rhesus macaques, the primary AIDS animal model. Here, we have shown that the human and AGM TRIM5α proteins are also able to block infection by a series of unrelated retroviruses and that TRIM5α is the likely identity of genes previously described as Ref1 in humans and Lv1 in monkeys. Fig. 1 shows that expression of either human or AGM TRIM5α in unrestricting cat cells confers on them the ability to restrict MLV-N but not the very closely related MLV-B. The degree of restriction, 2–3 logs for the human allele and 1 log for the AGM allele, is comparable with restriction of MLV-N in human and AGM cell lines, respectively (23). It seems likely that the slight restriction of MLV-B in cells expressing human TRIM5α is due to overexpression. Certainly overexpression of the murine restriction factor Fv1 B has been shown to expand its specificity, leading to a small restriction of MLV-B (30). Cat cells are extremely permissive for retrovirus infection and it seems that simply expressing exogenous TRIM5α from an unrelated species leads to the expected restriction phenotype of the TRIM5α host.

The ability of human and AGM TRIM5α to restrict retroviral infection is confirmed by the ability of siRNA to TRIM5α to restore infectivity of restricted virus in nonpermissive cells. Transfection of siRNA into human cells restores infectivity of MLV-N to titers equivalent to MLV-B, but unrestricted MLV-B titer is only slightly affected (Fig. 2). Furthermore, transfection of TRIM5α siRNA into AGM COS7 cells restores infectivity to a range of restricted viruses, including MLV-N as well as HIV-1

and EIAV. The titer of MLV-N is restored to almost that of MLB-B, but the unrestricted MLV-B titer is unaffected. The titer of HIV-1 and EIAV is increased by ≈10-fold, in agreement with the strength of the blocks as defined by experiments where Lv1 (TRIM5α) is saturated by restriction-sensitive virus-like particles (10, 11, 25).

The identification of Ref1/Lv1 as TRIM5α in humans and AGM has allowed us to examine the functional relationship between TRIM5α and the gag-like murine Fv1 restriction factor. Stable expression of Fv1 N in the human cell line TEN renders these cells able to restrict MLV-B in addition to their natural ability to restrict MLV-N (17). Remarkably, disruption of TRIM5α expression in TEN cells rescues the infectivity of both MLV-N and MLV-B, indicating a reliance of Fv1 N, in human cells, on endogenous TRIM5α protein expression (Fig. 4). This intriguing observation may begin to explain the identical specificity of TRIM5α and Fv1 for MLV-N amino acid CA110. If Fv1 requires TRIM5α for activity, then it is perhaps less surprising that the specificity of both is determined by the same amino acid. We postulate that Fv1 acts to modify the specificity of endogenous restriction factors, likely to be, or related to, TRIM5α in mice. This model could also explain previous data showing that overexpression of Fv1 B in a murine cell line endogenously expressing Fv1N renders the cells restrictive for MLV-N (the new allele works) but permissive for MLV-B (the endogenous allele no longer works) (30). This result could be explained by the exogenously expressed Fv1 B competing out the endogenous Fv1 N for a limited pool of murine TRIM5α. The endogenous Fv1 N would then be rendered inactive. This loss of restriction of MLV-N does not occur if Fv1 N is overexpressed in human cells because the Fv1 N simply expands the specificity of TRIM5α to MLV-B without affecting its intrinsic anti-MLV-N activity (17).

Fig. 3 demonstrates that down-regulation of TRIM5α expression in human cells rescues the ability of MLV-N to reverse transcribe. Fv1 blocks infection after reverse transcription. Why Fv1 restriction might block infection after reverse transcription and TRIM5α block before reverse transcription is unclear. However, the interaction between Fv1 and the incoming core is likely to occur at the same pre-reverse transcription step of infection because a polymerase negative mutant is perfectly able to saturate restriction. In other words, viral DNA synthesis is not required for Fv1-virus interaction. We imagine that interaction between sensitive virus and TRIM5α leads to a catastrophic event that prevents DNA synthesis whereas ternary interaction between Fv1, TRIM5α, and core leads to a catastrophic event realized only after reverse transcription.

The ability of arsenic trioxide to increase retroviral permissivity of human cells is specific for viruses that have been shown to be restricted by Ref1, namely MLV-N and EIAV. Unrestricted MLV-NB is unaffected by arsenic treatment (29)(Fig. 5). Remarkably the insensitivity of restriction in simian cells to arsenic is explained by differences between the human and simian TRIM5α proteins themselves, as shown by TRIM5α expression in murine cells. We assume that arsenic trioxide is able to specifically degrade or deregulate human TRIM5α activity by a mechanism related to the deregulation of the TRIM5α-related protein PML, possibly involving sumoylation (35). Whether other tripartite motif proteins are also modulated by arsenic trioxide remains unclear, as does the exact mechanism of the activity of this drug (34). Previous data showing an increase of HIV-1 reverse transcription and consequently an increase in HIV-1 titer on human cells, after arsenic trioxide treatment, is likely to be explained by a weak TRIM5α restriction of HIV-1 in some human cell lines (29, 36). The involvement of PML in HIV-1 permissivity remains unclear. Interactions between PML and incoming retrovirus, seen by immunofluorescence (36), is intriguing, and further characterization of the PML

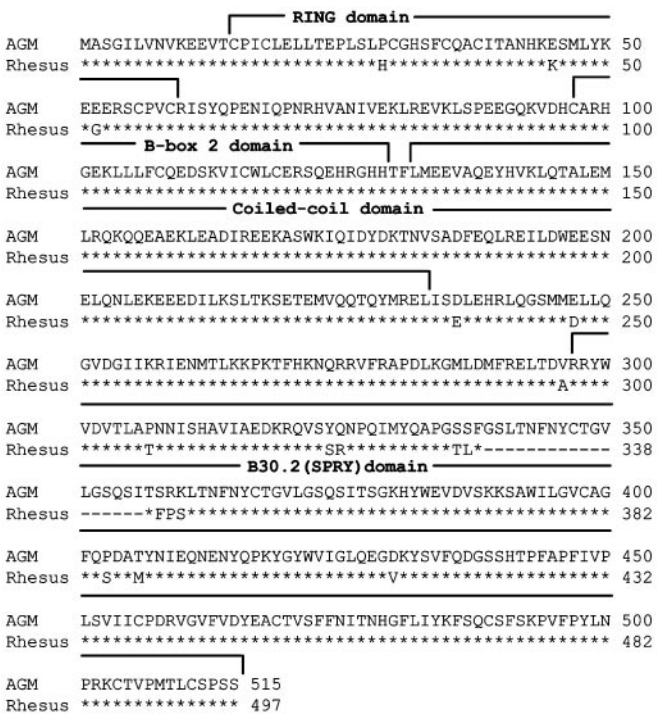

Fig. 6. Comparison of protein sequence of TRIM5α from rhesus macaque (*Macacca mulatta*) and AGM (*Cercopithicus* sp.) deduced from the cDNA sequence.

virus complex may be warranted. Certainly, the identification of restriction factors related to PML will focus attention on these observations once more.

The mechanism of retroviral restriction by saturable factors such as Fv1 and TRIM5α remains unsolved. The presence of a ubiquitin ligase domain in the TRIM5α protein suggests that ubiquitination and the consequent inactivation/degradation of incoming cores may be central to restriction factor activity. Understanding how specificity is controlled is likely to depend on analysis of TRIM5α alleles from different species and their specificity. The comparison between AGM and macaque TRIM5α proteins is striking and likely to be key. Our AGM TRIM5α sequence is very closely related to the macaque sequence but contains an 18-aa insertion in the B30.2 SPRY domain (Fig. 6) (37). The AGM TRIM5α seems to have broader specificity for viral CAs than does macaque TRIM5α. AGM cells are able to restrict MLV-N, HIV-1, HIV-2, simian immunodeficiency virus macaque (SIVmac), and EIAV compared with macaque cells strongly restricting only HIV-1, although this apparent broader specificity may be a consequence of the range of viruses tested. It will be interesting to examine the role of the SPRY domain sequence in its restriction specificity. It is intriguing that a SPRY domain, also found in the Ig superfamily, is found in TRIM5α, a protein able to protect against pathogens from within a cell.

Regarding restriction specificity, we are mindful of the fact that Fv1 specificity is controlled by point mutations in the virus and small changes between the Fv1 alleles themselves but is nonetheless complex (18, 30, 38). A single residue also controls HIV-1 restriction in owl monkey cells (26). Apobec3G species specificity can also be controlled by a single amino acid in the simian apobec3G protein (39). There is much evidence to suggest that individuals might be differentially permissive to viral infection (40, 41). It will be interesting to examine the role of TRIM5α polymorphism in intra-species variability of viral permissivity in humans as well as other mammals. The proposed involvement of the tripartite protein PML in control of herpes

virus replication (42, 43) suggests that this work should extend beyond retroviral permissivity.

We thank Alberto Fraile-Ramos, Srinika Ranasinghe, Ben Webb, Yasuhiro Ikeda, Yasuhiro Takeuchi, Sam Wilson, Edward Tsao, and Robin Weiss (University College London); Guangxia Gao (Chinese Academy of Sciences, Beijing); and Andrew Yueh and Steve Goff (Columbia University, New York) for reagents, helpful discussion, and encouragement. This work was funded by Wellcome Trust Career Development Fellowship 064257 (to G.J.T.) and a University College London Graduate School Fellowship and an Overseas Research Student award (to Z.K.).

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