Commentary

An intracellular block to primate lentivirus replication

Jonathan P. Stoye*

Division of Virology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

Vertebrates and retroviruses have coexisted for tens of millions of years as evidenced by the fossil record provided by endogenous retroviruses (1, 2). One might therefore predict that a degree of coevolution would have occurred resulting in the generation of host mechanisms for restricting virus spread as well as the appearance of relatively apathogenic viruses. Indeed, a number of cell-autonomous host defenses against retroviruses have been described. They include mutations of cellular receptors (3) and coreceptors (4), the expression of factors down-regulating or blocking receptors (5) as well as genes interfering with intracellular steps in viral replication (6, 7). Analysis of these genes and their modes of action, as well as the identification and subsequent characterization of novel genes with anti-retroviral activity, would be expected both to shed further light on retrovirus replication and suggest novel targets for therapeutic intervention. The latest World Health Organization (WHO) projected mortality figures for the AIDS epidemic emphasise the need for novel insights into HIV type 1 (HIV-1) replication and factors controlling its pathogenicity. Two papers in this issue of PNAS, by Cowan *et al.* (8) and Besnier *et al.* (9), represent an important step in this direction.

For a number of years it has been known that different primates differ in their susceptibility to infection, with HIV-1 showing a relatively narrow host range *in vivo* compared with most simian immunodeficiency viruses (SIVs). This restricted host range is mirrored by *in vitro* infection studies using peripheral blood mononuclear cells (PBMC). Early studies showed that SIVmac, a strain that was isolated from infected rhesus macaques, but that originated in sooty mangabeys and is closely related to HIV-2 (10), could replicate on both monkey and human PBMC, whereas HIV-1 grew only on human cells (11). HIV-1 replication in monkey PBMC is blocked at an early postentry, preintegration stage of the viral life cycle (12). The capsid (CA)-p2 domain of the viral Gag protein determines replication potential (13). A recent study using cells taken from a variety of different tissues and monkeys has confirmed and extended these results revealing further host range patterns (14). Some species (mainly New World monkeys) yield cells preferentially infected by HIV-1 compared with SIVmac, whereas others (mainly, but not exclusively, Old World monkeys) showed the reverse pattern. In a few cells lines HIV-1 and SIVmac grow to very low titres, implying a block affecting both viruses. Infectivity appears to be species- rather than tissue-specific, suggesting the involvement of widely expressed, genetically determined cellular factors in controlling host tropism.

One unresolved issue from the earlier studies was the nature of this block. Failure to replicate might be caused by the presence of one or more inhibitory factors in the infected cells. Alternatively, it might result from the absence of some cellular factor(s) absolutely required for virus replication. The papers by Cowan *et al.* (8) and Besnier *et al.* (9) address this question; both provide compelling evidence in favor of one or more dominant inhibitory factor(s) binding to incoming virions, thereby preventing reverse transcription. Both groups demonstrate that restriction can be overcome by use of high multiplicities of infection. Both groups show that restriction can be overcome, or abrogated, by preinfection with large amounts of the restricted, but not a nonrestricted, virus. For example, if rhesus monkey cells (restrict HIV-1 but not SIVmac) are pretreated with large amounts of an HIV-1 packaged vector encoding resistance to puromycin, and then infected with an identical vector carrying a different marker like enhanced green fluorescent protein (EGFP), a marked increase in GFP positive cells will be seen compared with control or SIV pretreated cells. Interestingly, in the one monkey cell line, CV1, which showed restriction of both HIV-1 and SIVmac, cross-abrogation was also observed. The abrogating particle must be capable of extracellular virus maturation associated with proteolysis of the intact Gag precursor polyprotein, i.e., must carry the Gag and Pro proteins but need not be capable of reverse transcription, in other words, it need not carry a functional Pol protein. Finally, Cowan *et al.* (8) showed that restriction is dominant in heterokaryons made between permissive and nonpermissive cells.

The genomes of a variety of primate species encode a saturable inhibitor of primate lentivirus infection.

Taken together, these data strongly support the notion that the genomes of a variety of primate species encode a saturable inhibitor of primate lentivirus infection that targets the CA and/or p2 components of the incoming subviral particle. This inhibitor closely resembles a mouse factor called Fv1 (Friend virus susceptibility 1) (15) so Cowan *et al.* (8) now propose to call the new activity Lv1. Further progress in understanding its mode of action appears dependent on identification of the gene encoding the activity. In the meantime it is perhaps instructive to compare the properties of Lv1 with those of Fv1 and a recently described nonmurine counterpart of Fv1 called REF1 (Resistance Factor 1) (16).

The properties of the three different restriction systems are summarized in Table 1. *Fv1* is a gene found only in mice (6). It has three major restricting alleles, n, nr, and b (17), defined by their ability to restrict different N- or B-tropic murine leukemia viruses (MLVs). REF1, found in a variety of nonmurine vetebrates including humans, restricts only N-MLV (16). Lv1 has three distinguishable activities, against HIV-1 or SIVmac or SIVmac and HIV-1, and it remains to be seen whether they correspond to three different alleles of one genetic locus or represent three separable genes. Indeed, it is possible that REF1 corresponds to an additional allele of Lv1 (see below). It will be interesting to see whether Lv1 acts on SIV isolates other than SIVmac.

The primary determinant of Fv1 specificity maps to CA amino acid 110 (18), although a number of neighboring amino acids can affect the Fv1-CA interaction (A. Stevens and J.P.S., unpublished data). CA amino acid 110 also determines the CA-REF1 interaction. Interestingly, this amino acid would be predicted (W. Taylor

See companion articles on pages 11914 and 11920.

*E-mail: jstoye@nimr.mrc.ac.uk.

COMMENTARY

Table 1. Properties of the three different restriction systems

Property	Fv1	REF1	Lv1
Activities*	1 → B-MLV 2 → B-MLV some N-MLV 3 → N-MLV	→ N-MLV	1 → HIV-1 2 → SIVmac 3 → HIV + SIVmac
Viral target	CA 110	CA 110	CA-p2
Site of action	After RT	Before RT	Before RT
Abrogation	Yes, by restricted virus	Yes, by restricted virus	Yes, by restricted virus (needs Gag cleavage)
Distribution	Mouse	Mainly primates	Primates
Gene	Related to MERV-L	?	?

*Distinguishable targets. For Fv1, 1–3 correspond to the alleles *Fv1*^{n}, *Fv1*^{nr}, and *Fv1*^{b}. RT, reverse transcription.

and J.P.S., unpublished data) to lie in the vicinity of the cyclophilin A binding site of HIV-1 CA (19). Thus, although the Lv1 binding site on HIV-1 CA/p2 remains to be mapped, it is tempting to speculate that it lies near that for cyclophilin A.

The most clear-cut phenotypic difference between *Fv1* and REF1/Lv1 apparently lies in the stage of the viral life cycle in which they act. Fv1 appears to act somewhat later (after reverse transcription) than REF1 (16) and Lv1 (before reverse transcription), at a stage in the viral life cycle where HIV-1 has already shed CA. However, it is not inconceivable that initial binding of Fv1 occurs at the same point in viral replication as REF1/Lv1 but that its phenotypic effect is manifested later. It should be noted that Fv1 is normally expressed in tiny amounts in cells (ref. 6, and M. Yap and J.P.S., unpublished data), an observation that may have important ramifications, both mechanistically and practically for the further study of REF1 and Lv1.

Restriction by Fv1/REF1/Lv1 is saturable, with abrogation occurring upon preinfection with restricted but not with nonrestricted virus. Lv1 abrogation requires Gag cleavage, suggesting that the interacting domain on CA/p2 is masked on the precursor protein, and implying that the interaction can only occur with incoming virions but not with virions in the process of exiting the cell. This would provide an explanation for the old observation that MLV producing cells are fully Fv1 protected (20). These observations would also suggest that unprocessed Gag cannot be used as bait for isolating REF1/Lv1 either directly or by two hybrid approaches. Indeed, cloned Fv1 and MLV CA do not appear to interact in the yeast two-hybrid system (S. Goff, personal communication).

Recent cross-abrogation studies, using two cell lines derived from African Green monkeys, provide a tantalizing clue that REF1 and Lv1 may be related (P. Bieniasz, personal communication). These cells inhibit N-MLV but not B-MLV, i.e., are REF1 positive, as well as SIVmac and HIV-1 via Lv1. Preinfection with HIV-1 increases titres of N-MLV 100-fold without affecting B-MLV replication. N-MLV, but not B-MLV, shows significant (but not complete) abrogation of HIV-1 restriction. These observations suggest the same factor is binding N-MLV as HIV-1, implying that REF1 is an allele of Lv1. If this is true, then Lv1 would have at least four alleles directed against HIV-1 (most Old World monkeys), SIVmac (New World monkeys), HIV-1, SIVmac and N-MLV (African Green monkeys), and N-MLV (humans). Given the differences in the CA protein of the different viruses, this finding raises considerable difficulty in explaining restriction based on the Fv1 model in which a single amino acid provides the basis for viral specificity.

Fv1 is approximately 60% identical at the nucleotide level to the *gag* gene of the endogenous retroviruses HERV-L and MERV-L (6, 21). One discernible feature is the predicted presence of a motif (Q-X3-E-X7-R), called the Major Homology Region, that is present in the CA protein of all retroviruses (21). Its precise role is unknown, but it is clearly important for virus assembly and/or entry of newly infected cells (22). Mutation of the conserved amino acids in the Fv1 Major Homology Region abolishes restriction (23), suggesting that it is functioning in a CA-like manner. Importantly, there is virtually no sequence similarity to its viral target. This result raises the possibility that resistance to lentiviral infection might be caused by one or more members of the virtually limitless pool of nonlentiviral endogenous retroviruses present in the genomes of primates (24). If this is the case, it is likely that the different activities of Lv1 represent independent genes rather than representing different alleles of the same gene.

Further progress in this area appears dependent on the molecular identification of REF1 and Lv1. This may not prove a trivial undertaking, particularly if the genes are expressed only at low levels and their products act only on the mature forms of CA. Fv1 was cloned only through positional means (6). Further genetic information about REF1 and Lv1 would be very helpful because it would help to define the number of genes in question and might suggest possible candidates. Expression cloning by transduction of cDNA libraries followed by selection of cells that show resistance to infection, an approach that has been used to clone a resistance factor to MLV from rat cells (28) seems to be the most promising approach, but carries no guarantee of success. Provided that REF1 is expressed in somatic cell hybrids, analysis of a suitable radiation hybrid panel might provide a rapid means of gene identification (25).

Over the next few years it appears likely that naturally occurring host mechanisms providing defense against retroviral infection will come under increasing scrutiny in attempts to further understand host–pathogen interactions. The recently cloned cellular gene that inactivates HIV-1 in the absence of the viral Vif protein (26) will undoubtedly prove one focus of this work as will *Fv1* and its cousins. More of these genes will likely be found. Moreover, the dozens of interactions that take place between virus and host proteins after viral infection (27) will also be examined in the hope of developing new approaches to treating viral disease.

I thank John Coffin and Melvyn Yap for helpful comments.

1. Goodchild, N. L., Wilkinson, D. A. & Mager, D. L. (1993) *Virology* **196,** 778–788.
2. Boeke, J. D. & Stoye, J. P. (1997) in *Retroviruses*, eds. Coffin, J. M., Hughes, S. H. & Varmus, H. E. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 343–435.
3. Levy, J. A. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 802–804.
4. Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., M. E. MacDonald, Stuhlmann, H., Koup, R. A. & Landau, N. R. (1996) *Cell* **47,** 367–378.
5. Taylor, G. M., Gao, Y. & Sanders, D. A. (2001) *J. Virol.* **75,** 11244–11248.
6. Best, S., Le Tissier, P., Towers, G. & Stoye, J. P. (1996) *Nature (London)* **382,** 826–829.
7. Simon, J. H. M., Gaddis, N. C., Fouchier, R. A. M. & Malim, M. H. (1998) *Nat. Med.* **4,** 1397–1400.
8. Cowan, S., Hatziioannou, T., Cunningham, T., Muesing, M. A., Gottlinger, H. G. & Bieniasz, P. D. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 11914–11919.
9. Besnier, C., Takeuchi, Y. & Towers, G. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 11920–11925.
10. Gao, F., Yue, L., White, A. T., Pappas, P. G., Barchue, J., Hanson, A. P., Greene, B. M., Hansen, A. P., Greene, B. M., Sharp, P. M., *et al.* (1992) *Nature (London)* **358,** 495–499.
11. Shibata, R., Kawamura, M., Sakai, H., Hayami, M., Ishimoto, A. & Adachi, A. (1991) *J. Virol* **65,** 3514–3520.
12. Shibata, R., Sakai, H., Kawamura, M., Tokunaga, K. & Adachi, A. (1995) *J. Gen. Virol.* **76,** 2723–2730.

13. Dorfman, T. & Göttlinger, H. G. (1996) *J. Virol.* **70,** 5751–5757.
14. Hofmann, W., Schubert, D., LaBonte, J., Munson,L., Gibson, S., Scammell, J., Ferrigno, P. & Sodroski, J. (1999) *J. Virol.* **73,** 10020–10028.
15. Jolicoeur, P. (1979) *Curr. Top. Microbiol. Immunol.* **86,** 67–122.
16. Towers, G., Bock, M., Martin, S., Takeuchi, Y., Stoye, J. P. & Danos, O. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 12295–12299.
17. Steeves, R. & Lilly, F. (1977) *Annu. Rev. Genet.* **11,** 277–296.
18. Kozak, C. A. & Chakraborti, A. (1996) *Virology* **225,** 300–306.
19. Gamble, T. R., Vajdos, F. F., Yoo, S., Worthylake, D. K., Houseweart, M., Sundquist, W. I. & Hill, C. P. (1996) *Cell* **87,** 1285–1294.
20. Duran-Troise, G., Bassin, R. H., Wallace, B. F. & Rein, A. (1981) *Virology* **112,** 795–799.
21. Bénit, L., de Parseval, N., Casella, J.-F., Callebaut, I., Cordonnier, A. & Heidmann, T. (1997) *J. Virol.* **71,** 5652–5657.
22. Craven, R. C., Leure-duPree, A. E., Weldon, R. A., Jr., & Wills, J. W. (1995) *J. Virol.* **69,** 4213–4227.
23. Bishop, K. N., Bock, M., Towers, G. & Stoye, J. P. (2001) *J. Virol.* **75,** 5182–5188.
24. Tristem, M. (2000) *J. Virol.* **74,** 3715–3730.
25. Rai, S. K., DeMartini, J. C. & Miller, A. D. (2000) *J. Virol.* **74,** 4698–4704.
26. Sheehy, A. M., Gaddis, N. C., Choi, J. D. & Malim, M. H. (2002) *Nature (London)*; published online before print July 14, 2002, 10.1038/nature00939.
27. Greene, W. C. & Peterlin, B. M. (2002) *Nat. Med.* **8,** 673–680.
28. Gao, G. & Goff, S. (2002) *Science*, in press.