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Mouse model to study the replication of primate foamy viruses

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A mouse model was developed to study the virus–host interaction of molecularly cloned human foamy virus (HFV) *in vivo*. The infectious process was analysed in two mouse strains, CBA/Ca and C57BL/6J, over a period of 24 weeks by PCR on DNAs from various animal tissues; virus serology was examined by immunoblotting. The infection persisted in both mouse strains and did not induce clinical symptoms. Upon infection of adult CBA/Ca mice HFV became detectable by PCR in an increasing number of organs over time. In contrast, in C57BL/6J mice, after an initial phase of dissemination, viral DNA sequences were found only in a few organs. Interestingly, the different course of infection was accompanied by differences in the antiviral immune response. In particular, C57BL/6J mice were high responders with respect to antibodies to the viral Bet protein, while CBA/Ca mice were low responders.

In vitro studies on foamy viruses (FVs) have disclosed some interesting properties of this group of retroviruses (for reviews see Rethwilm, 1995, 1996). However, there have been only a few investigations on the interaction of FVs with the living host which use modern molecular biology techniques (von Laer *et al.*, 1996; Saib *et al.*, 1997). Appropriate animal studies could reveal interesting information concerning the nature of FV target cells, viral gene expression, requirements of accessory genes for virus replication, the mechanism of virus persistence and the role of the immune system in counteracting virus replication. There are no suitable animal models, based on inbred rodent strains and molecularly cloned virus, for other complexly regulated primate retroviruses. We therefore established a mouse model to study the replication of FVs *in vivo* using the so-called human FV isolate (HFV2) (Schmidt *et al.*, 1997).

To determine whether rodents can be productively infected with primate FV, we infected one litter of newborns and four adults of six mouse strains (C3H/He, CBA/Ca, C57BL/6J, DBA/1, DBA/2 and SJL) and six rat strains (DA, Lew, WKY/N, LE/Cpb, BN/SsN and PVG) (animals were purchased from Harlan and Winkelmann) with 10^6 infectious units of cell-bound HFV2 by intraperitoneal (i.p.) injection. At 4 and 8 weeks p.i. two animals from each group were sacrificed and analysed for the presence of FV. The spleens of individual animals were divided into two. One half was used for virus re-isolation by co-cultivation with MRC-5 cells and DNA was extracted from the other half for nested FV PCR using *pol* gene primers (see Fig. 1 legend). In addition, we tested the anti-HFV immune response by Western blotting (WB). Since we used cell-bound virus in this experiment a WB reaction was regarded as positive only if an immune response against the Tas/Bel-1 transactivator protein developed between 4 and 8 weeks p.i. Such antibodies developed in some rodent strains infected as neonates (DBA/1, C57BL/6J, BN/SsN and WKY/N) and in some strains infected as adults (DBA/1, SJL, C57BL/6J, BN/SsN, DA, WKY/N and Lew). All infected animals were found to be positive by PCR on spleen cell DNA (data not shown). Aseptically removed spleen cells (2×10^6 cells/ml) were co-cultivated after stimulation with 10 μg/ml lipopolysaccharide (Sigma) and 3 μg/ml concanavalin A (Pharmacia) with MRC-5 cells for 1 week. Fibroblasts were subcultured for an additional 3–7 weeks; however, HFV was recovered only occasionally from BN/SsN and DA rats infected as neonates and from one CBA/Ca mouse infected as an adult. Animals appeared to tolerate the infection well and no apparent signs of disease that could be attributed to FV infection were identified. This systematic test of susceptibility revealed that different strains of inbred rats and mice could be infected with molecularly cloned FV. We chose CBA/Ca and C57BL/6J mice for further analyses because of the different immune response to the virus in these two strains.

Using cell-free HFV2 (a freeze–thaw lysate of infected cells cleared by low-speed centrifugation and passed through a 0·45 μm filter), adult CBA/Ca and C57BL/6J mice were infected by the i.p. route with 10^6 infectious units and the course of infection was monitored over a period of 24 weeks. Six mice for each time-point (4, 8, 12 and 24 weeks p.i.) were analysed for the presence of FV DNA in different organs by

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A

	CBA				BL/6			
weeks p.i.	4	8	12	24	4	8	12	24
spleen	3	5	5	6	5	4	3	3
thymus	1	2	2	2	6	4	2	1
ovaries	1	1	2	6	5	4	3	3
lung	0	1	3	4	4	5	3	2
liver	0	0	1	1	1	3	3	2
CNS	1	0	0	2	2	1	0	0

B

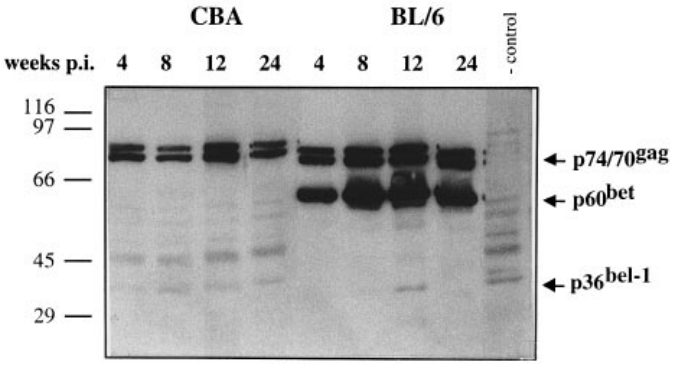

Fig. 1. Course of FV infection in CBA/Ca and C57BL/6J mice. Adult CBA/Ca and C57BL/6J mice were infected by the i.p. route with 10^6 infectious units of cell-free HFV2. At 4, 8, 12 and 24 weeks p.i., six animals of each strain were sacrificed, perfused (Schwender *et al.*, 1991) and analysed. (A) Detection of HFV DNA in different organs of infected animals by nested PCR using *pol*-gene-specific primers (#196, GCCACCCAAGGA-AGTTATGTGG; #197, GCTGCACCTTG-ATCAGAGTG; #198, CCTGGATGC-AGAGTTGGATC; #199, GAAGGAGC-CTTAGTGGGGTA). Organs were prepared individually and DNA was extracted using the Qiaamp tissue kit (Qiagen); 1 µg of DNA was used for the amplification by PCR. The conditions of amplification were essentially as described previously (Schweizer *et al.*, 1994, 1995). For the detection of positive results, half of the DNA was run on an ethidium bromide-containing 1·5% agarose gel and photographed. Of the six samples of each organ analysed, the number of samples that tested positive for HFV by PCR are given. (B) Typical antibody response of infected CBA/Ca and C57BL/6J mice determined by WB reaction on strips coated with HFV2-infected MRC-5 lysate (Hahn *et al.*, 1994). The migration of molecular mass markers is indicated on the left. Note that CBA/Ca mice did not produce detectable Bet antibodies.

PCR with *pol* gene primers, the development of an anti-FV immune response by WB and virus isolation from spleen cells. As shown in Fig. 1(A), we noted a marked difference between CBA/Ca and C57BL/6J mice in the detectability of FV DNA in different organs. In CBA/Ca mice after 4 weeks p.i. only a few animals tested positive, but over time the number of positive animals and the number of positive organs per animal increased. In C57BL/6J mice a different scenario was observed; the number of positive animals and organs was maximal in the early phase of infection (4 and 8 weeks p.i.) and then gradually declined.

Analysis of the anti-FV immune response (Fig. 1B) revealed that C57BL/6J mice mounted a strong anti-Gag and anti-Bet immune response as is usually observed by WB in primates naturally infected with FV (Netzer *et al.*, 1990; Hahn *et al.*, 1994; Rösener *et al.*, 1996). In contrast, adult CBA/Ca mice developed a weaker anti-FV Gag immune response and had no detectable Bet antibodies. Only one CBA/Ca mouse infected for 24 weeks was positive for virus isolation. However, this result provided further evidence that HFV infection persisted.

Analysis of CBA/Ca and C57BL/6J mice, infected with the cell-free virus as neonates, at 4 and 24 weeks p.i. revealed that, using *bel* gene primers, FV DNA could be detected in the vast majority of animals and organs at both time-points (Table 1). Consistent with the result obtained with animals infected as adults, CBA/Ca mice developed a clearly weaker anti-FV immune response compared with that observed in C57BL/6J mice, as determined by WB (Table 1). Gag antibodies were detected 24 weeks after infection in four CBA/Ca mice, one of which also showed Bet and Tas/Bel-1 antibodies. At this time-point, all infected C57BL/6J mice had built up a strong immune response against all three FV proteins. However, compared with C57BL/6J mice infected as adults, the development of an anti-Bet immune response in infected neonates was not accompanied by a restriction of the virus infection. An immature immune system leading to an initial lag phase before

Table 1. Course of HFV2 infection in newborn CBA/Ca and C57BL/6J mice

Twelve newborn CBA/Ca and C57BL/6J mice were each infected by the i.p. route with 10^6 infectious units of cell-free HFV2. Six mice of each strain were sacrificed and analysed after 4 and 24 weeks. The numbers indicate positive results per six animals per organ.

		CBA/Ca		C57BL/6J	
	Weeks p.i ...	4	24†	4	24
PCR*	Spleen	4	5	4	3
	Thymus	4	5	4	4
	Ovaries	6	5	4	6
	Lung	5	5	4	6
	Liver	6	5	4	5
	CNS	1	0	3	0
WB	α-Gag	2	4	5	6
	α-Bet	0	1	4	6
	α-Tas/Bel-1	0	1	0	6

* Nested PCR was performed using the *bel*-gene-specific primers (#293, AAATCCTCGACGCCCCAGACGATA; #294, CGAGGC-TTGTGATGCTTTTCAAAC; #295, AGTGAGCTTGTTGGC-CCTGAAAAT; #296, GTTTGGTAGGTTGCTGGACTCTTC) as described in the Fig. 1 legend.
† One CBA/Ca animal was WB negative and PCR negative in all tested organs.

FV antibodies develop and high cell division rates in the infected neonates may be responsible for this. No infectious virus was re-isolated from animals infected as neonates.

Some CBA/Ca and C57BL/6J mice infected as adults or as neonates were kept for a period of 50 weeks or longer. When spleen cell DNAs were analysed by PCR, persistent HFV infection could be demonstrated in the majority of the animals (9 out of 13) (data not shown).

We have shown recently that the HFV isolate has undergone deletions in the U3 region of the long terminal repeat (LTR) upon long-term replication in human diploid fibroblast cell cultures (Schmidt *et al*., 1997). At least one of the deletion variants (HSRV1) appeared to be replication competent *in vivo* in an accidentally infected human. We therefore wanted to know how the HFV LTR behaved in persistently infected mice. To investigate the replication competence of FVs with LTR deletions we infected adult CBA/Ca and C57BL/6J mice by the i.p. route with 10^6 infectious units of cell-associated: (i) HFV2; (ii) HSRV2; (iii) a virus mixture consisting of HFV2 (10%) and HSRV2 (90%); or (iv) HFVwt from 1985, consisting mainly of the HSRV1 variant and trace amounts of HFV2 (Schmidt *et al*., 1997). As shown in Table 2, all viruses were able to infect the two mouse strains. Analysis of spleen cell DNA with the LTR primers revealed that the dominant virus in the infecting pool (HSRV2 in the HSRV2/HFV2 mixture and HSRV1 in HFVwt) was also the dominant variant in the infected mice.

In some studies, rabbits and mice have already been used as infection models for HFV and simian foamy viruses (SFVs) (Johnston, 1974; Swack & Hsiung, 1975; Hooks & Detrick-Hooks, 1979; Brown *et al*., 1982; Santillana-Hayat *et al*., 1993; Saib *et al*., 1997). While Hooks & Detrick-Hooks (1979) used rabbits for infection with the chimpanzee isolate SFV-7 to investigate a transient suppression of cellular immunity, Brown *et al*. (1982) reported the persistent asymptomatic infection of outbred Swiss-Webster white mice with the chimpanzee virus SFV-6. In the latter study, a high frequency of SFV-6 re-isolations from explanted spleenic cultures in the initial phase of infection was reported. In none of these studies was molecularly cloned virus used.

The frequency of virus re-isolation was very low in all our experiments (HFV was re-isolated from 2 out of a total of 52 infected adult CBA/Ca mice). Differences in the genetic background of the mice, the methods used for virus isolation, and the use of a defined molecularly cloned virus instead of a more heterogeneous wild-type virus may explain the different results between our study and that of Brown *et al*. (1982). However, for virus detection, the PCR technique was evidently a good substitute for virus isolation.

Mice transgenic for HFV have been reported to develop a fatal encephalopathy and myopathy (for a review see Aguzzi *et al*., 1996). Clinical signs of disease were not seen in mice infected with HFV either as adults or as newborns. The lack of HFV neurotropism after experimental i.p. infection and the anti-HFV immune response even after infection of newborns, which is not observed in HFV transgenic mice, may explain this discrepancy.

Three molecular clones of HFV which are replication competent in cell culture have been characterized recently (Schmidt *et al*., 1997). These viruses differ in the lengths of their LTR U3 elements. Only the pHFV2 clone represents the complete HFV genome, while pHSRV1 and 2 are non-random U3 deletion variants. Such LTR deletion variants are not found in naturally infected chimpanzees; however, the HSRV1 virus was dominant, if not exclusively present, in an accidentally infected human (Schmidt *et al*., 1997). Consistent with this, all three viruses were able to infect CBA/Ca and C57BL/6J mice. Furthermore, when the inoculum consisted of a mixture of viruses with different U3 lengths, the dominant virus in the inoculum became the dominant virus *in vivo*. However, future studies may reveal more subtle *in vivo* differences between LTR-deleted and full-length virus, e.g. with respect to organ and cell tropism.

Although apes and monkeys are the natural hosts of primate FVs, our study suggests that a mouse model (CBA/Ca and C57BL/6J mice infected with HFV) can be used to investigate various aspects of the FV–host interaction. The function of the Bet protein is of prime interest. Bet is not strictly required for replication of HFV in cell-culture although

Table 2. Detection of HFV U3 deletion variants in spleens of mice infected with different HFV molecular clones

Four groups of six adult CBA/Ca and C57BL/6J mice were infected with 10^6 infectious units of four different cell-bound virus preparations. HFV2 represents the full-length HFV infectious molecular clone (Schmidt *et al.*, 1997). HSRV2 is the molecular clone of a naturally occurring 646 bp U3 deletion variant (Schmidt *et al.*, 1997; Schmidt & Rethwilm, 1995). HSRV2/HFV2 is a mixture of both viruses containing 90% infectious units of HSRV2 and 10% infectious units of HFV2. HFVwt is derived from a wild-type passage stored frozen since 1985. This virus contains mainly the HSRV1 510 bp U3 deletion variant and trace amounts of full-length virus (Schmidt *et al.*, 1997). Spleen cell DNAs of six animals of each strain per virus and per time-point were analysed by nested PCR using LTR-specific primers (Schmidt *et al.*, 1997) as described in the Fig. 1 legend. The numbers indicate the number of samples, out of the six analysed, that were positive.

		CBA/Ca		C57BL/6J	
Infecting virus	Weeks p.i ...	4	12	4	12
HSRV2		5	6	1	5
HFV2		0	6	2	3
HSRV2/HFV2 (90%/10%)		4/0	5/0	4/0	6/0
HFVwt (1985) (HSRV1/HFV2)		5/0	6/0	5/0	5/0

a minor effect on the development of virus titres in acute and persistent infection has been described for Bet$^-$ viruses (Baunach *et al.*, 1993; Yu & Linial, 1993; Schmidt & Rethwilm, 1995). The different course of infection observed in adult CBA/Ca and C57BL/6J mice was accompanied by a different antibody response against the Bet protein. This suggests that the Bet protein may have a role in efficient virus spread in the infected host and/or that an immune reaction against Bet may counteract virus spread. However, other immunological parameters, such as the development of neutralizing antibodies, which were not analysed in the current study, may also contribute to the differences in the course of infection between the two mouse strains. The availability of the mouse model described here, of mutated infectious molecular clones (Baunach *et al.*, 1993) and of HFV genes expressed by recombinant vaccinia viruses (Fischer *et al.*, 1997) should allow dissection of the infectious process of HFV in the mouse in detail.

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