Mouse model to study the replication of primate foamy viruses

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Abstract

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.
Mouse model to study the replication of primate foamy viruses

Martina Schmidt,1 Stefan Niewiesk,1 Jonathon Heeney,2 Adriano Aguzzi3 and Axel Rethwilm1

1 Institut für Virologie und Immunobiologie, Versbacher Str. 7, 97078 Würzburg, Germany
2 Laboratory of Viral Pathogenesis, Biomedical Primate Research Center (BPRC), Rijswijk, The Netherlands
3 Institut für Neuropathologie, Department Pathologie, Universitätsspital Zürich, Switzerland

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 106 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 × 106 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 105 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

Author for correspondence: Axel Rethwilm.
Fax +49 931 201 3934. e-mail rethwilm@vim.uni-wuerzburg.de
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; Rosener 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...
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 $10^8$ 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.

<table>
<thead>
<tr>
<th></th>
<th>CBA/Ca</th>
<th>C57BL/6J</th>
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<tbody>
<tr>
<td></td>
<td>4 24†</td>
<td>4 24</td>
</tr>
<tr>
<td>PCR*</td>
<td></td>
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<tr>
<td>Spleen</td>
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<tr>
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<tr>
<td>Liver</td>
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<tr>
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</tr>
<tr>
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<td></td>
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<tr>
<td>α-Gag</td>
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<td></td>
</tr>
<tr>
<td>α-Bet</td>
<td>0 1 4 6</td>
<td></td>
</tr>
<tr>
<td>α-Tas/Bel-1</td>
<td>0 1 0 6</td>
<td></td>
</tr>
</tbody>
</table>

* Nested PCR was performed using the bel-gene-specific primers (#293, AAATCTCTGACGCCCCAGACGATA; #294, CAGGCC-TTGATGCTTTTCAAAC; #295, AGTGAGCTTGTTGGCATGCTT; #296, GCTGAAAAT; #297, AAATCCTCGACGCCCCAGACGATA; #298, GCTGAAAAT) as described in the Fig. 1 legend. † One CBA/Ca animal was WB negative and PCR negative in all tested organs.

Some CBA/Ca and C57BL/6J mice 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).

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

<table>
<thead>
<tr>
<th>Infecting virus</th>
<th>CBA/Ca</th>
<th>C57BL/6J</th>
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<tbody>
<tr>
<td>HSRV2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>HFV2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>HSRV2/HFV2 (90% / 10%)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>HFVwt (1985)</td>
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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.

References


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