The effect of latency-associated transcript on the herpes simplex virus type 1 latency-reactivation phenotype is mouse strain-dependent

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Herpes simplex virus type 1 (HSV-1) latency-associated transcript (LAT) null mutants reactivate poorly in the rabbit ocular model. The situation in mice is less clear. Reports concluding that LAT null mutants reactivated poorly in the mouse explant-induced reactivation (EIR) model are contradicted by a similar number of reports of normal EIR of LAT− mutants in mice. To determine if the EIR phenotype might be mouse strain-dependent we infected BALB/c and Swiss Webster mice with LAT− or LAT+ virus and assessed EIR in individual trigeminal ganglia. Compared to LAT+ virus, LAT− virus reactivated poorly in Swiss Webster mice \( (P < 0.05) \). In contrast, the EIR phenotype of these viruses was similar in BALB/c mice \( (P > 0.1) \). Thus, LAT appeared to have a much greater impact on the EIR phenotype in Swiss Webster mice than in BALB/c mice. The mouse strain therefore appeared consequential in the HSV-1 EIR phenotype in mice.

Following ocular herpes simplex virus type 1 (HSV-1) infection, the virus travels up nerves and establishes life-long latent infection in nuclei of sensory neurons of the trigeminal ganglia (TG). At various times the virus may reactivate, travel back to the eye, and produce recurrent disease. This can produce scarring of the cornea and loss of vision, making recurrent HSV-1 a major cause of infectious corneal blindness in the developed world (Nesburn, 1983). A similar HSV latency-reactivation-recurrent disease cycle produces recurrent ‘cold sores’ in and around the mouth and recurrent genital lesions.

Latency-associated transcript (LAT) is the only viral gene abundantly transcribed during HSV-1 neuronal latency. In the rabbit ocular model LAT null mutants consistently have reduced in vivo reactivation (Bloom et al., 1994; Perng et al., 1994, 1996; Trousdale et al., 1991). In contrast, in the mouse model of explant-induced reactivation (EIR) of TG, the effect of LAT on reactivation is less clear. At least three reports indicate that mutants unable to transcribe LAT reactivated similarly to LAT+ virus in the mouse (Cook et al., 1991; Deshmene et al., 1993; Javier et al., 1988). Several other mutants thought at the time to disrupt LAT function also did not alter the EIR phenotype in mice (Block et al., 1990; Ho & Mocarski, 1989; Izumi et al., 1989; Junejo & Brown, 1995; Natarajan et al., 1991). In contrast, in other reports LAT mutants had significantly reduced reactivation in the mouse (Block et al., 1993; Devi-Rao et al., 1994; Leib et al., 1989; Sawtell & Thompson, 1992; Steiner et al., 1989). To add to the confusion, in at least two instances LAT mutants with normal EIR phenotypes in mice (Maggioncalda et al., 1994, 1996) were later shown by the same investigators to have significantly reduced reactivation in the rabbit (Hill et al., 1996, 1997).

LAT can enhance the efficiency of establishing latency (Perng et al., 2000a, b; Thompson & Sawtell, 1997), which may account for some of the effect of LAT on reactivation. Note that in this report we use the term ‘reactivation’ and ‘explant-induced reactivation’ (EIR) as a phenotype, much like ‘blue eyes’. Thus, even if the ability of LAT to increase EIR was completely due to the ability of LAT to enhance the efficiency of establishing latency (Perng et al., 2000a, b; Thompson & Sawtell, 1997), we would still say that LAT enhances reactivation. For clarity the term ‘reactivation phenotype’ or ‘EIR phenotype’ will be used below when appropriate.

To investigate the above discrepancies regarding LAT in the mouse EIR phenotype, we infected BALB/c and Swiss Webster mice with LAT− or LAT+ virus. We report here that in BALB/c mice no significant differences were seen in the EIR phenotype. In contrast, in Swiss Webster mice the EIR phenotype of the LAT− virus was significantly decreased.
dLAT2903, the LAT− virus used here, contains a 1.8 kb deletion in both copies of LAT (Perng et al., 1994). The deletion removes the primary TATA box-based LAT promoter and a second putative promoter located just prior to the stable 2 kb LAT region. The deletion also encompasses the first 1.67 kb of the primary 8.3 kb LAT and almost 1 kb of the 2 kb LAT. This mutant makes no detectable LAT transcripts, yet replicates in tissue culture, rabbit eyes and rabbit TG in a manner indistinguishable from the parental McKrae virus or marker-rescued dLAT2903R (Perng et al., 1994). In rabbits dLAT2903 has a reduced reactivation phenotype (Perng et al., 1994).

BALB/c mice and Swiss Webster mice were ocularly infected with 10^2, 10^3, 10^4, or 10^5 p.f.u./eye of LAT− or LAT+ virus, without corneal scarification. Because of expected differences in survival rates at different infectious doses, five
Effect of LAT on HSV-1 in mice

**Fig. 2.** Replication in eyes. Mice were ocularly infected without corneal scarification. Tear films were collected with nylon swabs at various times post-infection and the amount of virus was determined by plaque assay, as we have previously described (Perng et al., 1994). Each point represents the average titre from five eyes, each from a different mouse. Panel (A): BALB/c mice infected with 10^5 p.f.u./eye; (B) Swiss Webster mice infected with 10^5 p.f.u./eye; (C) BALB/c mice infected with 10^6 p.f.u./eye; (D) Swiss Webster mice infected with 10^6 p.f.u./eye. E, LAT^-; D, LAT^+.

mice/group were infected with the 10^2 and 10^3 p.f.u./eye doses, 10 mice/group were infected with the 10^4 p.f.u./eye dose, and 25 mice/group were infected with the 10^5 and 10^6 p.f.u./eye doses. TG were removed 30 days post-infection for determination of the kinetics of EIR.

Since more Swiss Webster mice than BALB/c mice survived (see below), some of the TG from the Swiss Webster mouse groups were randomly eliminated from the study such that the numbers of TG in each Swiss Webster mouse group were the same as the corresponding BALB/c mouse group. Thus any apparent differences in EIR between the BALB/c and Swiss Webster mice should not be due to unequal statistical power.

The cumulative percentages of TG that reactivated during the 18 day observation period are shown in Fig. 1(A–H). No significant reactivation was detected in either BALB/c or Swiss Webster mice following infection at 10^8 p.f.u./eye (Fig. 1I, J). In BALB/c mice infected with 10^2 (Fig. 1A) and 10^4 (Fig. 1D) p.f.u./eye, reactivation of the LAT^- and LAT^+ viruses was virtually identical. At 10^4 and 10^6 p.f.u./eye (Fig. 1B, C), the reactivation of LAT^- appeared slightly reduced compared to LAT^+ in the BALB/c mice, but the differences were not significant. Thus, as summarized in Fig. 1(I), at all doses there was no significant difference in EIR of the LAT^- and LAT^+ viruses from TG of BALB/c mice (P > 0.1).

In contrast, in Swiss Webster mice EIR of the LAT^- virus was significantly reduced compared to the LAT^+ virus at all infectious doses from 10^3 to 10^6 p.f.u./eye (Fig. 1E–H; summarized in Fig. 1J). These results are consistent with those we previously reported for dLAT2903 in the rabbit (Perng et al., 1994).
Fig. 3. Survival of BALB/c and Swiss Webster mice following ocular infection with LAT\(^-\) or LAT\(^+\) virus. Panels (A)–(E), mice were infected in both eyes with the indicated dose of virus and survival was followed for 21 days. The numbers over each bar indicate the no. of surviving mice/no. of mice infected. \(P\) values determined by chi-squared, are shown within each infectious dose only for those results that were significantly different (groups being compared are indicated by the lines above the bars).

Panels (F)–(I), additional analyses of the results shown in (A)–(E). \(P\) values are based on paired Student’s \(t\)-tests comparing infectious doses 10\(^3\)–10\(^6\) in (F); 10\(^4\)–10\(^6\) in (G); 10\(^3\)–10\(^5\) in (H); and 10\(^3\)–10\(^4\) in (I).

BALB/c mice and Swiss Webster mice were ocularly infected with 10\(^5\) or 10\(^6\) p.f.u./eye of LAT\(^-\) or LAT\(^+\) virus. Tears were collected at various times post-infection and virus was quantified by standard plaque assays as previously described (Perng et al., 1994) (Fig. 2). At 10\(^5\) p.f.u./eye the peak titres for these viruses were similar in BALB/c mice (Fig. 2A, day 5, \(P = 0.42\)) and in Swiss Webster mice (Fig. 2B, day 5 LAT\(^+\) compared to day 3 LAT\(^-\) \(P = 0.20\)). At 10\(^6\) p.f.u./eye, both viruses had similar replication kinetics and peak titres in the eyes of BALB/c mice (Fig. 2C; \(P = 0.1\) and \(P = 0.4\) for day 5 and 7 respectively) and Swiss Webster mice (Fig. 2D; \(P = 0.11\) for day 5). There was also no significant difference in peak virus titres of LAT\(^-\) and LAT\(^+\) in TG of BALB/c or Swiss Webster mice (not shown). These results are similar to our previous findings with these viruses in rabbits (Perng et al., 1994). Although peak virus titres were similar in the eyes of both mouse strains, the titres appeared to remain high for an extended time in eyes of BALB/c mice. This may be related to the decreased survival of BALB/c mice compared to Swiss Webster mice shown below.

Survival was followed for 21 days post-infection in the mice used for the above EIR studies (Fig. 3). Compared to BALB/c mice, Swiss Webster mice appeared to be more resistant to lethal ocular challenge with LAT\(^+\) (Fig. 3D, E, F; \(P = 0.01\) and \(P = 0.02\), chi-squared, for 10\(^5\) and 10\(^6\) p.f.u./eye respectively; \(P = 0.009\), paired Student’s \(t\)-test for the range 10\(^3\) to 10\(^6\) p.f.u./eye) and LAT\(^-\) virus (Fig. 3G, \(P = 0.02\) paired \(t\)-test over the range 10\(^3\) to 10\(^6\) p.f.u./eye). Although the results in Fig. 3 (H, I) show a trend for LAT\(^-\) virus to be less virulent (i.e. increased survival) than the LAT\(^+\) virus, especially at lower infectious doses, the differences were not significant (\(P > 0.05\) at all individual infectious doses and by paired \(t\)-test). This is consistent with our previous findings that the LAT\(^+\) and LAT\(^-\) viruses have similar virulence in rabbits (Perng et al., 1994).

As discussed above, there is a lack of consistency in the
literature regarding the effect of LAT on the HSV-1 EIR phenotype in mice. The results presented here may help to explain this discrepancy. Our findings suggest that in some mouse strains LAT has a major impact on the EIR phenotype, while in other mouse strains LAT has limited or no impact. Thus, differences in the mouse strains used to test LAT mutants may partially account for discrepancies in the literature. In addition, in BALB/c mice infected at 10^4 or 10^5 p.f.u./eye there appeared to be a nonsignificant tendency towards decreased EIR with the LAT^- virus, while at 10^6 p.f.u./eye there was no hint of any difference between the LAT^- and LAT^+ viruses. Thus, if LAT does play a role in the EIR phenotype in BALB/c mice, the effect may be dependent on infectious dose, with 10^6 p.f.u./eye being too high a dose. Since infectious doses in the range of 10^4 to 10^6 p.f.u./eye are typically employed in HSV-1 mouse experiments, even small differences in the infectious doses used in different studies may also partially account for the discrepancies in the literature. In addition, since the difference between LAT^- and LAT^+ in BALB/c mice approached significance at infectious doses of 10^4 and 10^6 p.f.u./eye, it is possible that, by chance, statistical significance might be achieved in some experiments but not in others, especially if small numbers of mice are used. All of these factors may contribute to the lack of consistency in the literature regarding the effect of LAT on the EIR phenotype in mice.

To determine if the findings presented here, i.e. that LAT was required for wild-type levels of EIR from Swiss Webster mouse TG but not BALB/c TG, are consistent with previous reports, we re-examined the literature. We found five publications in which apparently well-defined, molecularly constructed, LAT null mutants (i.e. mutants that were constructed such that they are deleted for the LAT promoter, with or without deletion of adjacent regions) were reported to have reduced or delayed EIR phenotypes in mice (Block et al., 1993; Devi-Rao et al., 1994; Leib et al., 1989; Sawtell & Thompson, 1992; Steiner et al., 1989). In three of these papers (Devi-Rao et al., 1994; Leib et al., 1989; Sawtell & Thompson, 1992) the studies were done in Swiss Webster mice or CD-1 mice, which, like Swiss Webster mice, are an outbred strain. These reports are therefore consistent with the results reported here. The remaining two studies were done in BALB/c mice (Block et al., 1993; Steiner et al., 1989). However, the mutants used in both of these studies had unexpected genetic defects in addition to the LAT deletion. In one case the LAT^- virus made small plaques and grew poorly (Block et al., 1993). Neither of these phenotypes have been reported in any other defined LAT^- virus. In the other case (Steiner et al., 1989), the LAT^- virus was later shown to be defective for gC also (Wroblewska et al., 1991).

Of the three reports suggesting that LAT^- viruses have EIR phenotypes similar to that of LAT^+ virus, one was done in BALB/c mice (Deshmane et al., 1993), and this is consistent with the results we reported here. The other two were done in Swiss Webster mice. However, both of these studies used the LAT^- mutant × 10-13 (Cook et al., 1991; Javier et al., 1988). This virus was fortuitously derived from an HSV-1 × HSV-2 intertypic recombinant (Javier et al., 1987) during the course of marker rescue experiments in mouse brain following intracranial inoculation (Javier et al., 1988). Thus, in addition to containing HSV-2 sequences, × 10-13 may contain numerous unknown alterations. In addition, in one of the × 10-13 studies, no marker-rescued LAT^- virus was used, EIR was not studied kinetically, and the EIR was done with dorsal root ganglia, not TG (Javier et al., 1988). In the other study, the number of mice was very small, only six per group (Cook et al., 1991). This study reported no statistical significance between explant reactivation of the LAT^- (× 10-13) virus and the LAT^+ virus, with a P value of 0.09. It is possible that statistical significance would have been reached had a larger number of mice been used. Thus, consistent with the results reported here, all of the truly well-defined LAT^- viruses that were reported to have decreased EIR phenotypes in mice were studied in either CD-1 or Swiss Webster mice, and all of the truly well-defined LAT^- viruses reported to have normal EIR phenotypes in mice were studied in BALB/c mice.

The results reported here, combined with the above survey of the literature, suggest that in contrast to other mouse strains, rabbits and presumably humans, in BALB/c mice the presence or absence of LAT has little effect on the EIR phenotype of HSV-1 from TG. The genetic difference in BALB/c mice that is responsible for this remains to be determined.

In summary, the results reported here suggest that in Swiss Webster mice it is easy to detect the effect of LAT on the EIR phenotype, while in BALB/c mice such an effect is difficult to observe and, if present, may be highly dependent on initial infectious dose of the viruses.

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