Viral replicative capacity is the primary determinant of lymphocytic choriomeningitis virus persistence and immunosuppression

Andreas Bergthaler,a,b,1, Lukas Flatz,a,b,1, Ahmed N. Hegazy,b,c, Susan Johnson,a,d Edit Horvathb, Max Löhnib,c, and Daniel D. Pinschewera,b,d,2

*aDepartment of Pathology and Immunology, Centre Médical Universitaire, University of Geneva, 1211 Geneva, Switzerland; bInstitute of Experimental Immunology, Department of Pathology, University Hospital Zurich, 8091 Zurich, Switzerland; cExperimental Immunology, Department of Rheumatology and Clinical Immunology, Charité–University Medicine Berlin, German Rheumatism Research Center, 10117 Berlin, Germany; and dWorld Health Organization Collaborating Centre for Neonatal Vaccinology, University of Geneva, 1211 Geneva, Switzerland

Edited* by Rolf M. Zinkernagel, University Hospital Zurich, Zurich, Switzerland, and approved October 22, 2010 (received for review August 12, 2010)

The Clone 13 (Cl13) strain of lymphocytic choriomeningitis virus is widely studied as a model of chronic systemic viral infection. Here, we used reverse genetic techniques to identify the molecular basis of Cl13 persistence and immunosuppression, the characteristics differentiating it from the closely related Armstrong strain. We found that a single-point mutation in the Cl13 polymerase was necessary and partially sufficient for viral persistence and immunosuppression. A glycoprotein mutation known to enhance dendritic cell targeting accentuated both characteristics but when introduced alone, failed to alter the phenotype of the Armstrong strain. The decisive polymericase mutation increased intracellular viral RNA load in plasmacytoid dendritic cells, which we identified as a main initial target cell type in vivo, and increased viremia in the early phase of infection. These findings establish the enhanced replicative capacity as the primary determinant of the Cl13 phenotype. Viral persistence and immunosuppression can, thus, represent a direct consequence of excessive viral replication overwhelming the host’s antiviral defense.

persistent viral infections | viral polymerase | plasmacytoid dendritic cell | viral tropism | acquired immunodeficiency syndrome

Systemic persistent infection with lymphocytic choriomeningitis virus (LCMV) has been studied for almost a century (1), providing important concepts of virus–host interaction that subsequently have been extended to HIV and hepatitis C virus (HCV) infection in humans (2). One particularly useful feature of the LCMV model is the existence of virus strains that are genetically closely related but differ in their ability to establish persistent infection in vivo (3, 4). Thereby, self-limiting infection (successful immune response) and persistent infection (unsuccessful immune defense, which is often associated with acquired immunodeficiency) can be compared side by side to investigate underlying mechanisms.

Much progress has recently been made to understand the version of the antiviral immune defense in the context of persistent infection. Signaling through inhibitory receptors such as programmed death 1 (5) and lymphocyte-activation gene 3 (6) and their respective ligands contributes to the establishment of persistent infection by limiting immune responses (7, 8). Thereby, self-limiting infection (successful immune response) and persistent infection (unsuccessful immune defense) can be compared side by side to investigate underlying mechanisms.

Viral replicative capacity of specific viral isolates.

HIV as well as HCV can replicate in DCs (22–26). Moreover, the parameters governing persistence or clearance of hepatitis C and B virus remain incompletely defined (27–29). Hence, a better general understanding of these mechanisms in murine LCMV infection may have general implications for the development of refined strategies for the prevention of chronicity of viral infections.

Results

Reverse Genetic Mapping of LCMV Cl13 Persistence. Using reverse genetic tools (30), we set out to map the genetic basis of Cl13 persistence. We revisited the ARM and Cl13 genome sequences (GenBank numbers AY847350, AY847351, DQ361065, and DQ361066), which revealed coding and noncoding mutations as outlined in Tables 1 and 2 and Fig. 1. Three coding changes were identified (denominated as ARM → Cl13). F → L at amino acid 260 of the viral GP (GP260L) is known to account for increased receptor binding affinity (19). Furthermore, we found a previously unknown N → D mutation at amino acid 176 of GP (GP176D) and K → Q at amino acid 1,079 of the viral polymerase gene L (L1079Q). This latter change is commonly found in persistence-prone LCMV variants (19, 32). Noncoding mutations


The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

1A.B. and L.F. contributed equally to this work.

2To whom correspondence may be addressed. E-mail: andreas.bergthaler@gmail.com or daniel.pinschewer@gmx.ch.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1011998107/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1011998107

PNAS | December 14, 2010 | vol. 107 | no. 50 | 21641–21646

ROU
BHK-21 cells used to amplify viral stocks. This was not unex-
ected because of the known identical growth kinetics of ARM and Cl13 in standard immortalized cell lines (34).

For in vivo mapping of the Cl13 persistence phenotype, we infected C57BL/6 mice with either of the recombinant viruses. The course of viremia (Fig. 1B) displayed a clear segregation of the 16 viruses into three main phenotypic clusters, indicative for an unbalanced contribution of the four mutations to the pheno-
typic difference of ARM and Cl13. Viruses with the L1079 poly-
merase version of Cl13 (C/X/X/X) reached considerably higher peak viremia than A/X/X/X viruses, indicating that this mutation dictates virus load differences between ARM and Cl13. Within the group of eight C/X/X/X viruses, the four C/C/X/X viruses persisted longer than the four C/A/X/X viruses, which is compatible with the known role of GP260 in facilitating DC targeting. However, infection with A/C/X/X viruses resulted in viremia of similarly short duration as A/A/X/X infections, suggesting that the GP260-mediated DC targeting effect required the L1079 muta-
tion. The course of viremia (Fig. 1A) clearly demonstrates the importance of ARM and Cl13. Viruses with the L1079 poly-
merase version of Cl13 (C/X/X/X) reached considerably higher peak viremia than A/X/X/X viruses, indicating that this mutation dictates virus load differences between ARM and Cl13. Within the group of eight C/X/X/X viruses, the four C/C/X/X viruses persisted longer than the four C/A/X/X viruses, which is compatible with the known role of GP260 in facilitating DC targeting. However, infection with A/C/X/X viruses resulted in viremia of similarly short duration as A/A/X/X infections, suggesting that the GP260-mediated DC targeting effect required the L1079 mutation.

Genetic Determinants of LCMV-Induced CD8⁺ T-Cell Exhaustion and Generalized Immunosuppression. Next, we studied whether differ-
ential virus loads and persistence correlated with the propensity of the various recombinant viruses to subvert the LCMV-speci-
cific CTL response, a process commonly referred to as exhaustion (4, 10, 11). For this, we determined the frequency of IFN-γ and TNF-
α-producing CD8⁺ T cells specific for nucleoprotein (NP) 396–404 (NP396) and GP33–41 (GP33) on day 22 after infection (Fig. 2 A and B). NP-specific CTLs are of particular importance for early virus control (35) and are most affected by exhaustion, whereas GP-specific cells are more resistant to this process (11). The NP396-specific response of animals infected with viruses carrying the L1079 version of ARM (A/X/X/X viruses) was signifi-
cantly higher than the one of mice harboring C/X/X/X viruses, indicating that the Cl13 polymerase mutation was also the primary determinant of suppressed LCMV-NP-specific CTL responses. Notably, C/C/X/X (GP260 of Cl13) and C/A/X/X viruses (GP260 of ARM) caused similar suppression of NP396-specific CTL responses, whereas GP33-specific responses were only suppressed in C/C/X/X-infected mice (L1079 and GP260 of Cl13). Irre-
versible of the epitope studied, A/C/X/X-infected animals (GP260 of Cl13) exhibited a similarly intact CD8⁺ T-cell response as those infected with A/A/X/X viruses (GP260 of ARM), indicating that GP260 of Cl13 failed to promote CTL exhaustion in the absence of
LCMV-GP33-specific CD8+ (%)

Table 2. Comparison of the LCMV-ARM with LCMV-Cl13 genomes: L segment

<table>
<thead>
<tr>
<th>Nucleotide position*</th>
<th>416</th>
<th>1,965</th>
<th>3,963</th>
<th>5,269</th>
<th>5,962</th>
<th>6,325</th>
<th>6,559</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic element</td>
<td>IGR</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>Amino acid change</td>
<td>–</td>
<td>–</td>
<td>K1079 → Q1079</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ARM (nt)†</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Cl13 (nt)§</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>G</td>
<td>T</td>
</tr>
</tbody>
</table>

*Counted from the first nucleotide of 5′ UTR as position 1.
†LMCV-ARM L segment sequence: GenBank AY847351 (31).
§LMCV-Cl13 L segment sequence: GenBank DQ361066 (30).

†Not applicable; located in IGR (noncoding cis-acting RNA element).

The L1079 mutation. To analyze the role of L1079 and GP260 in CD8+ T-cell exhaustion in spleen, we analyzed responses to a select set of viruses (X/X/A/A viruses) on day 13 after infection (Fig. 2 C and D). GP33-specific responses to viruses carrying the L1079 version of ARM (A/X/A/A viruses) were significantly higher than the one of mice harboring C/X/A/A viruses, indicating that the Cl13 polymerase mutation was also the primary determinant of splenic CD8+ T-cell responses to GP33 on day 13. Conversely, the introduction of the GP260 mutation did not exert obvious effects on GP33-specific responses, neither when tested in combination with L1079 (C/C/A/A vs. C/A/A/A) nor when assessed on its own (A/C/A/A vs. A/A/A/A). Analogous trends were noted for splenic NP396-specific responses, but these responses exhibited a higher degree of variability, and potential differences between groups failed to reach statistical significance. In summary, the analysis of LCMV-specific CTL exhaustion in spleen and blood revealed a very consistent role of the L1079 position, whereas the contributive effect of GP260 was more variable.

LCMV infection can also cause generalized immunosuppression, dampening the immune defense against unrelated third-party infections. To assess the viral genetic basis for immunosuppression, we infected mice with our panel of LCMV recombinants and subsequently challenged them with vesicular stomatitis virus (VSV). VSV infection elicits an early and potent neutralizing IgG response, preventing viral neuroinvasion and fatal myeloencephalitis (36, 37), and it elicits a potent CD8+ T-cell response to the dominant VSV NP-derived epitope NP52–59 (NP52). We performed VSV challenge on day 18 after LCMV infection and determined VSV-neutralizing IgG titers and NP52-specific CD8+ T-cell responses 8 d later (26 d after LCMV infection) (Fig. 2 C and D). These analyses revealed a similar hierarchy of generalized...
immunosuppression as observed for exhaustion of LCMV-specific CTL responses. Mice infected with C/C/X/X viruses (L1079 and GP260 of Cl13) mounted significantly lower VSV-specific IgG titers and VSV-NP52–specific CD8+ T-cell responses than animals infected with C/A/X/X, A/C/X/X, or A/A/X/X viruses. These results established a joint role of the Cl13 mutations L1079 and GP260 in suppressing humoral and cellular immune responses to an unrelated third-party antigen. Interestingly, GP260 of Cl13 and thus, enhanced DC targeting (19) failed to significantly accentuate the suppression of VSV-specific immune responses when tested in the context of the ARM polymerase (similar responses in A/C/X/X- and A/A/X/X-infected mice; P > 0.05). Conversely, VSV-neutralizing IgG responses were lower in infected animals with C/A/X/X viruses than in A/A/X/X-infected groups (P < 0.01), indicating that the polymerase position L1079 by itself enhanced the immunosuppressive capacity of LCMV independently of the GP260 mutation. Taken together, these results established the L1079 position of Cl13 as the primary determinant of peak viremia and generalized immunosuppression. Additionally, the GP260 mutation, which is responsible for increased α-dystroglycan affinity and DC targeting, played an accessory role in enhancing the duration of persistence and generalized immunosuppression by those viruses that also carried the L1079 position of Cl13.

**C113 Polymerase Mutation Enhances Replication in pDCs and Increases Early Viremia.** We then aimed to differentiate between the possibilities that the L1079 position directly affected the viral replicative capacity in vivo and that increased viral loads resulted solely from subverted and thus, inefficient immune defense. For this purpose, we analyzed viremia on day 4 (i.e., before the onset of the antiviral CD8+ T-cell response). Already at this early time point, higher levels of viremia than viruses carrying the ARM version of the polymerase (A/X/X/X viruses, P < 0.05) and thus, was indicative for L1079-Cl13 (lysine at position 1079 of L) and rLCMV/Cre-L1079-Cl13 vector genomes (glutamine at position 1079 of L). Note that substitution of GP for Cre recombinase renders the resulting vector replication-deficient. (C and D) STOP-GFP reporter mice were inoculated with 10^7 pfu of rLCMV/Cre-L1079-Cl13 pseudotyped with GP of Cl13 from producer cells; 3 d later, pDC and mDC subsets (discriminated according to mPDCA-1 and CD11c expression) were analyzed for GFP expression. rLCMV/OVA (vector expressing ovalbumin instead of Cre recombinase) served as GFP background control. Numbers indicate the percentage of GFP-positive cells. (D) STOP-GFP reporter mice were inoculated with 10^7 pfu of rLCMV/Cre_L1079-Cl13 or rLCMV/Cre_L1079-ARM. Both vectors were pseudotyped with the GP of Cl13 from producer cells to assure identical cell targetting; 3 d later, GFP-positive pDCs were sorted by FACS, total cellular RNA was extracted, and LCMV 5 segment RNA was determined by quantitative RT-PCR. Each symbol represents a pool of sorted cells prepared from splenocytes obtained from three mice.

Fig. 3. Cl13 polymerase mutation enhances replication in pDCs and increases early viremia. (A) C57BL/6 mice were infected with 2 × 10^6 pfu of the indicated viruses i.v., and viremia was determined 4 d later. Symbols represent individual mice. Representative results from one of two independent experiments are shown. (B) Schematic representation of the replication-deficient rLCMV/Cre_L1079-ARM (lysine at position 1079 of L) and rLCMV/Cre-L1079-Cl13 vector genomes (glutamine at position 1079 of L). Note that substitution of GP for Cre recombinase renders the resulting vector replication-deficient. (C and D) STOP-GFP reporter mice were inoculated with 10^7 pfu of rLCMV/Cre_L1079-Cl13 pseudotyped with GP of Cl13 from producer cells; 3 d later, pDC and mDC subsets (discriminated according to mPDCA-1 and CD11c expression) were analyzed for GFP expression. rLCMV/OVA (vector expressing ovalbumin instead of Cre recombinase) served as GFP background control. Numbers indicate the percentage of GFP-positive cells. (D) STOP-GFP reporter mice were inoculated with 10^7 pfu of rLCMV/Cre_L1079-Cl13 or rLCMV/Cre_L1079-ARM. Both vectors were pseudotyped with the GP of Cl13 from producer cells to assure identical cell targetting; 3 d later, GFP-positive pDCs were sorted by FACS, total cellular RNA was extracted, and LCMV 5 segment RNA was determined by quantitative RT-PCR. Each symbol represents a pool of sorted cells prepared from splenocytes obtained from three mice.
infected pDCs than its ARM counterpart (rLCMV/Cre_L1079-ARM) resulted in revealed that the L gene of Cl13 (expressed by rLCMV/PCR and standardization for a housekeeping gene (Fig. 3) positive) pDCs by FACS sorting. Quanti
hence inoculated STOP-GFP mice with either rLCMV/Cre_L1079-ARM or Cl13 version of the L polymerase vectors, expressing either the ARM (rLCMV/Cre_L1079-ARM) or Cl13 version of the L protein (47). Still, several not mutually exclusive possibilities remain as to how L1079 affects the Cl13 immunobiology. Differential resistance of ARM and Cl13 replication to IFN has been reported (48) and may be related to the L1079 mutation. Also, polymerase processivity may be affected directly by influencing interactions of the L protein with cellular cofactors of viral replication and transcriptional complexes (51, 49). Differential availability of specific cellular cofactors and cell type-specific cofactor isoforms could, therefore, explain the differential impact of L1079 on viral replication in different cell types (50). Of note, L-protein mutations other than K1079Q can also enhance persistence of ARM (19, 42), spreading the infection to other cell types throughout the body. In line with this observation, a comparison of the GP260 and L1079 positions in several commonly used LCMV strains (Table S1) suggests that other strains such as Docile establish persistence, despite sharing the K1079 residue of the L protein with ARM.

Systemic persistent infections like HIV and HCV infections represent important global health problems. It is, therefore, of paramount importance that we understand better how viruses establish persistence, how they subvert the antiviral T-cell defense (51, 52), and which parameters of infection facilitate generalized immunosuppression. Using reverse genetic techniques with LCMV, we show here that a single-point mutation enhancing viral replication in the initial target cell population in vivo can dictate all of the above three parameters. Importantly, viral growth assessments in standard immortalized cell lines fail to detect these differences (34), despite wide-ranging effects on the viral immunobiology. The mechanisms governing persistence or clearance of hepatitis C and B virus infection are still poorly defined (27–29). Our present findings in a widely used model of systemic persistent infection suggest that isolate-specific differences in replicative capacity can profoundly influence the viral immunobiology, including the resulting antiviral defense of the host. Hence, such differences should be considered anew as potential strain- and isolate-specific determinants of hepatitis virus chronicity as well as a key pathogenic determinant in other persistent viral infections.

Methods

Mice and Animal Experiments. C57BL/6 and STOP-GFP mice (39) were bred under SPF conditions at the Institut für Labortierkunde, University of Zurich, Switzerland. Animal experiments were performed at the Universities of Zurich and Geneva with permission by the respective Cantonal Veterinary Office and in accordance with the Swiss law for animal protection.

Viruses, Virus Titration, and Assessment of Virus-Neutralizing Antibodies. LCMV Armstrong ARM5.3b (ARM), LCMV Cl13, and VSV have been described (30). The 1,079 position does not map to any of the known catalytic domains in the L protein (47). Still, several not mutually exclusive possibilities remain as to how L1079 affects the Cl13 immunobiology. Differential resistance of ARM and Cl13 replication to IFN has been reported (48) and may be related to the L1079 mutation. Also, polymerase processivity may be affected directly by influencing interactions of the L protein with cellular cofactors of viral replication and transcriptional complexes (51, 49). Differential availability of specific cellular cofactors and cell type-specific cofactor isoforms could, therefore, explain the differential impact of L1079 on viral replication in different cell types (50). Of note, L-protein mutations other than K1079Q can also enhance persistence of ARM (19, 42), spreading the infection to other cell types throughout the body. In line with this observation, a comparison of the GP260 and L1079 positions in several commonly used LCMV strains (Table S1) suggests that other strains such as Docile establish persistence, despite sharing the K1079 residue of the L protein with ARM.

Systemic persistent infections like HIV and HCV infections represent important global health problems. It is, therefore, of paramount importance that we understand better how viruses establish persistence, how they subvert the antiviral T-cell defense (51, 52), and which parameters of infection facilitate generalized immunosuppression. Using reverse genetic techniques with LCMV, we show here that a single-point mutation enhancing viral replication in the initial target cell population in vivo can dictate all of the above three parameters. Importantly, viral growth assessments in standard immortalized cell lines fail to detect these differences (34), despite wide-ranging effects on the viral immunobiology. The mechanisms governing persistence or clearance of hepatitis C and B virus infection are still poorly defined (27–29). Our present findings in a widely used model of systemic persistent infection suggest that isolate-specific differences in replicative capacity can profoundly influence the viral immunobiology, including the resulting antiviral defense of the host. Hence, such differences should be considered anew as potential strain- and isolate-specific determinants of hepatitis virus chronicity as well as a key pathogenic determinant in other persistent viral infections.

Methods

Mice and Animal Experiments. C57BL/6 and STOP-GFP mice (39) were bred under SPF conditions at the Institut für Labortierkunde, University of Zurich, Switzerland. Animal experiments were performed at the Universities of Zurich and Geneva with permission by the respective Cantonal Veterinary Office and in accordance with the Swiss law for animal protection.

Viruses, Virus Titration, and Assessment of Virus-Neutralizing Antibodies. LCMV Armstrong ARM5.3b (ARM), LCMV Cl13, and VSV have been described (30). The 1,079 position does not map to any of the known catalytic domains in the L protein (47). Still, several not mutually exclusive possibilities remain as to how L1079 affects the Cl13 immunobiology. Differential resistance of ARM and Cl13 replication to IFN has been reported (48) and may be related to the L1079 mutation. Also, polymerase processivity may be affected directly by influencing interactions of the L protein with cellular cofactors of viral replication and transcriptional complexes (51, 49). Differential availability of specific cellular cofactors and cell type-specific cofactor isoforms could, therefore, explain the differential impact of L1079 on viral replication in different cell types (50). Of note, L-protein mutations other than K1079Q can also enhance persistence of ARM (19, 42), spreading the infection to other cell types throughout the body. In line with this observation, a comparison of the GP260 and L1079 positions in several commonly used LCMV strains (Table S1) suggests that other strains such as Docile establish persistence, despite sharing the K1079 residue of the L protein with ARM.

Systemic persistent infections like HIV and HCV infections represent important global health problems. It is, therefore, of paramount importance that we understand better how viruses establish persistence, how they subvert the antiviral T-cell defense (51, 52), and which parameters of infection facilitate generalized immunosuppression. Using reverse genetic techniques with LCMV, we show here that a single-point mutation enhancing viral replication in the initial target cell population in vivo can dictate all of the above three parameters. Importantly, viral growth assessments in standard immortalized cell lines fail to detect these differences (34), despite wide-ranging effects on the viral immunobiology. The mechanisms governing persistence or clearance of hepatitis C and B virus infection are still poorly defined (27–29). Our present findings in a widely used model of systemic persistent infection suggest that isolate-specific differences in replicative capacity can profoundly influence the viral immunobiology, including the resulting antiviral defense of the host. Hence, such differences should be considered anew as potential strain- and isolate-specific determinants of hepatitis virus chronicity as well as a key pathogenic determinant in other persistent viral infections.

Methods

Mice and Animal Experiments. C57BL/6 and STOP-GFP mice (39) were bred under SPF conditions at the Institut für Labortierkunde, University of Zurich, Switzerland. Animal experiments were performed at the Universities of Zurich and Geneva with permission by the respective Cantonal Veterinary Office and in accordance with the Swiss law for animal protection.

Viruses, Virus Titration, and Assessment of Virus-Neutralizing Antibodies. LCMV Armstrong ARM5.3b (ARM), LCMV Cl13, and VSV have been described (30). The 1,079 position does not map to any of the known catalytic domains in the L protein (47). Still, several not mutually exclusive possibilities remain as to how L1079 affects the Cl13 immunobiology. Differential resistance of ARM and Cl13 replication to IFN has been reported (48) and may be related to the L1079 mutation. Also, polymerase processivity may be affected directly by influencing interactions of the L protein with cellular cofactors of viral replication and transcriptional complexes (51, 49). Differential availability of specific cellular cofactors and cell type-specific cofactor isoforms could, therefore, explain the differential impact of L1079 on viral replication in different cell types (50). Of note, L-protein mutations other than K1079Q can also enhance persistence of ARM (19, 42), spreading the infection to other cell types throughout the body. In line with this observation, a comparison of the GP260 and L1079 positions in several commonly used LCMV strains (Table S1) suggests that other strains such as Docile establish persistence, despite sharing the K1079 residue of the L protein with ARM.

Systemic persistent infections like HIV and HCV infections represent important global health problems. It is, therefore, of paramount importance that we understand better how viruses establish persistence, how they subvert the antiviral T-cell defense (51, 52), and which parameters of infection facilitate generalized immunosuppression. Using reverse genetic techniques with LCMV, we show here that a single-point mutation enhancing viral replication in the initial target cell population in vivo can dictate all of the above three parameters. Importantly, viral growth assessments in standard immortalized cell lines fail to detect these differences (34), despite wide-ranging effects on the viral immunobiology. The mechanisms governing persistence or clearance of hepatitis C and B virus infection are still poorly defined (27–29). Our present findings in a widely used model of systemic persistent infection suggest that isolate-specific differences in replicative capacity can profoundly influence the viral immunobiology, including the resulting antiviral defense of the host. Hence, such differences should be considered anew as potential strain- and isolate-specific determinants of hepatitis virus chronicity as well as a key pathogenic determinant in other persistent viral infections.

Methods

Mice and Animal Experiments. C57BL/6 and STOP-GFP mice (39) were bred under SPF conditions at the Institut für Labortierkunde, University of Zurich, Switzerland. Animal experiments were performed at the Universities of Zurich and Geneva with permission by the respective Cantonal Veterinary Office and in accordance with the Swiss law for animal protection.
Statistical Analysis. For comparison of results, we determined one-way ANOVA with Bonferroni's posttest. Two groups were compared by two-tailed Student t test. The analysis was carried out using GraphPad Prism software (vs. 4.0b). P values of P < 0.05 were considered statistically significant (*), and P = 0.01 was considered highly significant (**). P > 0.05 was considered not statistically significant (ns).

ACKNOWLEDGMENTS. A.B. is a European Molecular Biology Organization long-term fellow and was supported by a PhD fellowship of the Boehringer Ingelheim Fonds, postdoctoral scholarships of the Roche Research Foundation, and the Schweizerische Stiftung für Medizinisch-Biologische Stipendien of the Swiss National Science Foundation. A.N.H. was a fellow of GRK 1121 of the German Research Foundation. S.J. is a recipient of an Australian National Health and Medical Research Council postdoctoral training fellowship. M.L. is a Lichtenberg fellow funded by the Volkswagen Foundation. D.D.P. was supported by Grant 3100A-104067/1 of the Swiss National Science Foundation, the European Community seventh framework program (European Virus Archive Grant 228292), and the Prix Scientifique of the Foundation Lejeune Awards, and he holds a stipendary professorship from the Swiss National Science Foundation (PP00A–119413/1).
### Supporting Information

**Bergthaler et al. 10.1073/pnas.1011998107**

Table S1. Comparison of the glycoprotein 260 and long-segment 1,079 position in widely used lymphocytic choriomeningitis virus strains

<table>
<thead>
<tr>
<th>Gene and amino acid position*</th>
<th>GP&lt;sub&gt;260&lt;/sub&gt;</th>
<th>L&lt;sub&gt;1079&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCMV strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Armstrong&lt;sup&gt;†&lt;/sup&gt;</td>
<td>F</td>
<td>K</td>
</tr>
<tr>
<td>Clone 13&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>L</td>
<td>Q</td>
</tr>
<tr>
<td>WE&lt;sup&gt;§&lt;/sup&gt;</td>
<td>L</td>
<td>K</td>
</tr>
<tr>
<td>Aggressive&lt;sup&gt;¶&lt;/sup&gt;</td>
<td>L</td>
<td>K</td>
</tr>
<tr>
<td>Docile&lt;sup&gt;∥&lt;/sup&gt;</td>
<td>L</td>
<td>K</td>
</tr>
<tr>
<td>Pasteur&lt;sup&gt;**&lt;/sup&gt;</td>
<td>L</td>
<td>K</td>
</tr>
<tr>
<td>Traub&lt;sup&gt;††&lt;/sup&gt;</td>
<td>L</td>
<td>K</td>
</tr>
</tbody>
</table>

GP, glycoprotein; L, long segment; LCMV, lymphocytic choriomeningitis virus.

*Counted from the first amino acid of the respective ORF.

<sup>†</sup>GenBank AY847350 and AY847351.
<sup>‡</sup>GenBank DQ361065 and DQ361066.
<sup>§</sup>GenBank M22138 and AF004519.
<sup>¶</sup>GenBank EU480450 and EU480451.
<sup>∥</sup>GenBank EU480452 and EU480453.
<sup>**</sup>GenBank DQ868485 and DQ868486.
<sup>††</sup>GenBank DQ868487 and DQ868488.