Noncytopathic viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) in humans and lymphocytic choriomeningitis virus in mice may establish persistent infections. Therapeutic strategies that allow control or elimination of persisting virus infections by either vaccination or antiviral drug treatment are sought. The nucleoside analog ribavirin has been shown to exhibit a broad spectrum of antiviral activity (45, 47) by blocking the enzyme inosine monophosphate dehydrogenase and suppressing viral RNA synthesis (47). Treatment with ribavirin exhibited some benefit for patients suffering from Lassa fever, Argentine hemorrhagic fever, and hepatitis C (21, 22, 36, 37, 45–47). In experimental animal model infections, ribavirin has also been demonstrated to exhibit broad antiviral activities (7, 26, 28, 29, 53, 54).

The immune response against viruses which can lead to persistent infections is characterized by strong initial cytotoxic T-lymphocyte (CTL) responses followed by poor and delayed virus-neutralizing antibody responses (5, 14, 34, 35, 38, 48, 58, 61). In such infections it has been demonstrated that virus-neutralizing antibodies make a limited contribution to virus clearance (9, 27, 43, 44, 55).

Here we tested whether an early and accelerated virus-neutralizing antibody response or antiviral drug treatment or the combination of both can prevent a chronic infection. In the mouse, the natural host of lymphocytic choriomeningitis virus (LCMV), acute LCMV infection is controlled by CTLs (18, 30, 39, 62). Virus-neutralizing antibodies, which develop late after infection, are crucial for long-term control of LCMV (56) and beneficial in humans after infections with persistent viruses such as hepatitis C virus and hepatitis B virus and possibly human immunodeficiency virus.

Additive Effect of Neutralizing Antibody and Antiviral Drug Treatment in Preventing Virus Escape and Persistence

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Poorly cytopathic or noncytopathic viruses can escape immune surveillance and establish a chronic infection. Here we exploited the strategy of combining antiviral drug treatment with the induction of a neutralizing antibody response to avoid the appearance of neutralization-resistant virus variants. Despite the fact that H25 immunoglobulin transgenic mice infected with lymphocytic choriomeningitis virus mounted an early neutralizing antibody response, the virus escaped from neutralization and persisted. After ribavirin treatment of H25 transgenic mice, the appearance of neutralization-resistant virus was prevented and virus was cleared. Thus, the combination of virus-neutralizing antibodies and chemotherapy efficiently controlled the infection, whereas each defense line alone did not. Similar additive effects may be unexpectedly efficient and beneficial in humans after infections with persistent viruses such as hepatitis C virus and hepatitis B virus and possibly human immunodeficiency virus.

Noncytopathic viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) in humans and lymphocytic choriomeningitis virus in mice may establish persistent infections. Therapeutic strategies that allow control or elimination of persisting virus infections by either vaccination or antiviral drug treatment are sought. The nucleoside analog ribavirin has been shown to exhibit a broad spectrum of antiviral activity (45, 47) by blocking the enzyme inosine monophosphate dehydrogenase and suppressing viral RNA synthesis (47). Treatment with ribavirin exhibited some benefit for patients suffering from Lassa fever, Argentine hemorrhagic fever, and hepatitis C (21, 22, 36, 37, 45–47). In experimental animal model infections, ribavirin has also been demonstrated to exhibit broad antiviral activities (7, 26, 28, 29, 53, 54).

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Here we tested whether an early and accelerated virus-neutralizing antibody response or antiviral drug treatment or the combination of both can prevent a chronic infection. In the mouse, the natural host of lymphocytic choriomeningitis virus (LCMV), acute LCMV infection is controlled by CTLs (18, 30, 39, 62). Virus-neutralizing antibodies, which develop late after infection, are crucial for long-term control of LCMV (56) and have an important function in protection against reinfection (8, 57, 60). After infection with a high dose of LCMV strain

DOCILE, virus-specific CTLs may be exhausted; this results in a persistent LCMV infection of the host within 10 to 20 days (40). Transfer of immune sera into neonatal mice can contribute to the prevention of persistent infection (9, 10).

In contrast, in the absence of neutralizing-antibody responses, establishment of viral persistence is accelerated (13, 43, 56). H25 transgenic mice, which express the μ heavy chain of the LCMV-neutralizing monoclonal antibody (MAb) KL25, mount an early and accelerated LCMV-neutralizing antibody response comparable to an antibody response after an antiviral vaccination of nontransgenic mice (51). Earlier studies showed that such transgene-encoded virus-neutralizing antibodies enhanced virus clearance after low-dose infection with the immediately replicating LCMV strain WE. The neutralizing antibodies lowered the viral burden and thereby supported the CTL-mediated virus clearance (51). Similar effects have been observed after transfer of MAbs (9, 50). Here we show that after high-dose infection with the rapidly replicating LCMV strain DOCILE the enhanced virus-neutralizing antibody responses in H25 transgenic mice did not prevent virus persistence, which correlated with antibody escape variants emerging in vivo. However, additional treatment of H25 transgenic mice with the antiviral drug ribavirin together with the early LCMV-neutralizing antibody response prevented selection of LCMV antibody escape variants, and LCMV was cleared from ribavirin-treated H25 transgenic mice. Ribavirin treatment alone, in nontransgenic C57BL/6 mice, did not prevent LCMV persistence. Thus, the additive effect of virus-neutralizing antibodies and antiviral drug treatment prevented persistent virus infection by precluding immune escape of LCMV. These data suggest that similar additive effects may be unexpectedly efficient and beneficial in humans after infections with persistent viruses such as HCV, HBV, and HIV.

MATERIALS AND METHODS

Mice. H25 transgenic mice expressing the μ heavy chain of the LCMV-neutralizing MAb KL25 produce LCMV-neutralizing immunoglobulin M (IgM) antibodies early after LCMV infection (51). Sex- and age-matched C57BL/6 control mice were purchased from the Institut für Zuchthygiene, University Zurich. Mice were bred under specific-pathogen-free conditions, and experiments were performed under conventional conditions. Mice were treated intra-
peritoneally (i.p.) with 5 mg of ribavirin (Virazole ribavirin; ICN Farmaceutica, Iztapalapa, Mexico) daily for 2 weeks, and control mice were left untreated.

**Virus.** LCMV strain DOCILE was originally provided by J. Pfau, New York, N.Y., and was grown on BHK cells. LCMV antibody escape variants were tested as described previously (52). Briefly, loss of binding to the parental transgenic MAb KL25 was tested by fluorescence-activated cell sorter (FACS) surface staining of LCMV GP expressed on infected MC57G cells. Homogenous cell surface expression of LCMV GP by mutant and wild-type LCMV was confirmed by FACS staining with the MAb WEN1, which recognizes mutant and wild-type LCMV GP to the same extent. Loss of neutralization was tested in an infectious focus reduction assay (11). The presence of LCMV antibody escape mutants was confirmed by reverse transcription-PCR (RT-PCR) Taq cycle sequencing of LCMV GP (Taq Dye Deoxy terminator cycle sequencing kit; Applied Biosystem Inc.; Foster City, Calif.; Bio-Rad). RT-PCR was performed using LCMV GP1-specific primers as described previously (52). PCR products were sequenced by automated Taq cycle sequencing (Taq Dye Deoxy terminator cycle sequencing kit; Applied Biosystems Inc.; Bio-Rad).

**Nucleotide sequence accession numbers.** The sequences of the PCR products are available from the EMBL nucleotide sequence database under accession no. AJ249149 to AJ249159.

**RESULTS**

Early LCMV-neutralizing antibodies in H25 transgenic mice do not prevent LCMV persistence. In order to investigate the impact of an early LCMV-neutralizing antibody response on the development of a persisting infection with LCMV, we infected H25 transgenic mice expressing the μ heavy chain of the LCMV-neutralizing MAb KL25 (51) and nontransgenic C57BL/6 control mice intravenously (i.v.) with 5 × 10^4 PFU of LCMV DOCILE and analyzed neutralizing serum antibody titers and virus titers in different organs. Early after infection by day 4, H25 transgenic mice mounted a strong LCMV-neutralizing antibody response, whereas LCMV-infected control C57BL/6 mice did not exhibit any detectable virus-neutralizing activity (Fig. 1A). However, despite this early LCMV-neutralizing antibody response in H25 transgenic mice, LCMV established a persistent infection. Virus titers similar to those found in nontransgenic C57BL/6 LCMV carrier mice were detected in spleens, kidneys, blood, livers, and lungs of LCMV-infected H25 transgenic mice during the entire observation period of 120 days (Fig. 1B). Selection of LCMV antibody escape variants in H25 transgenic mice. To test whether virus from H25 transgenic mice had escaped the neutralizing-antibody response, virus was collected from blood at days 4, 8, and 13 after infection and used to infect MC57G mouse fibroblasts in vitro. After 40 h of culture, LCMV GP expressed on the surfaces of infected MC57G cells was analyzed by FACS for the binding of MAb KL25. As shown in Fig. 2A, cell surface-expressed LCMV GP of virus isolated from H25 transgenic mice and that isolated from C57BL/6 control mice at day 4 after infection were recognized by MAb KL25 to similar extents (Fig. 2A, graphs A and D). However, MAb KL25 recognized only part of the virus isolated from H25 transgenic mice at day 8 after infection (Fig. 2A, graph B) and no virus isolated from H25 transgenic mice at day 13 after infection (Fig. 2A, graph C), in contrast to results for virus isolated from nontransgenic C57BL/6 mice (Fig. 2A, graphs E and F). Control FACS analysis with MAb WEN1, which recognizes wild-type LCMV GP and the antibody escape variant of LCMV GP to the same extent (52), resulted in comparable staining of the cell cultures infected with day 13 virus isolates, demonstrating equally efficient LCMV GP cell surface expression by wild-type and variant viruses (Fig. 2A, graphs G and H). Similar results were obtained for virus isolated at days 4 and 8 after infection (data not shown). Thus, LCMV which persisted in H25 transgenic mice had escaped the neutralizing-antibody response.

This is in agreement with previous data demonstrating that LCMV persisting in neonatally infected mice in the presence of neutralizing antibodies are antibody escape variants (52) and with data demonstrating that a MAb neutralizing LCMV strain Armstrong A4 but not strain Armstrong A5 protected mice against infection with Armstrong A4 but not with Armstrong A5 (9). These two viruses had, however, been cloned from a laboratory stock; they were not the result of immune selection.

Generation of LCMV antibody escape variants was confirmed by RT-PCR cloning and Taq cycle sequencing of the LCMV GP: all LCMV isolated from H25 transgenic mice at day 13 after infection exhibited the amino acid substitution of LCMV GP Asn119, which has been shown (52) to be indicative
of escape from the KL25 antibody response (Fig. 2B, sequences H25-29.1 to H25-29.7). LCMV isolated from C57BL/6 control mice exhibited wild-type LCMV DOCILE sequences (Fig. 2B, sequences Ctrl-29.8 to Ctrl-29.10).

Additive effect of LCMV-neutralizing antibodies and ribavirin treatment in control of persistent viral infection in vivo. The antiviral drug ribavirin has been shown to interfere with LCMV replication in vitro (23). In order to test the effect of ribavirin on LCMV propagation in vivo, H25 transgenic mice and nontransgenic C57BL/6 mice were infected i.v. with \( 5 \times 10^4 \) PFU of LCMV DOCILE and were either treated i.p. with 5 mg of ribavirin daily for 2 weeks or were left untreated. Virus titers in spleen, kidney, liver, and lung were determined 13 days after infection. As shown in Fig. 3, H25 transgenic mice treated with ribavirin had no detectable virus titers in the organs tested. In contrast, ribavirin-treated C57BL/6 control mice lacking virus-neutralizing antibody titers exhibited high virus titers in all organs tested. Both untreated H25 transgenic mice and untreated C57BL/6 control mice had also not cleared LCMV infection, and the virus persisted at least until day 60 after infection (data not shown). Thus, only the additive effect of LCMV-neutralizing antibodies in H25 transgenic mice and ribavirin treatment permitted control of infection and viral clearance.

LCMV isolates from spleens, kidneys, and livers of non-ribavirin-treated H25 transgenic mice 11 days after the initial infection were identified as LCMV antibody escape variants. After infection of MC57G cells, surface-expressed LCMV GP did not bind MAb KL25 as revealed by FACS analysis (Fig. 4A to C). In contrast, LCMV isolated from ribavirin-treated H25 transgenic mice at day 11 after infection, i.e., 2 days before virus clearance, consisted of wild-type LCMV still recognized by MAb KL25 (Fig. 4D to F). Thus, ribavirin prevented the emergence of LCMV antibody escape variants in H25 transgenic mice, thereby enabling the neutralizing antibodies to efficiently contribute to the CTL-mediated viral clearance.
DISCUSSION

In the present study, an enhanced and accelerated LCMV-neutralizing antibody response in H25 transgenic mice did not prevent the establishment of a persistent LCMV infection. LCMV escaped the transgene-encoded neutralizing-antibody response by selection of viral antibody escape variants. Escape from the neutralizing-antibody response, however, was prevented by antiviral drug treatment. These data illustrate the subtle balances between virus kinetics and the host immune response and how the balance can be influenced to the advantage of the host by means of an antiviral drug.

Antibodies are effective in antiviral treatment and protection by passive or active vaccination. While very efficient against cytopathic viruses, an isolated antibody response alone often is not sufficient to control noncytopathic or poorly cytopathic viruses (9, 27, 43, 44, 55). For LCMV infection, neutralizing antibodies can prevent LCMV escape variants (8, 9, 43, 51). However, sometimes an isolated antibody response may be disadvantageous for the host because of the risk of antibody-mediated enhancement of disease (12, 25) or the emergence of viral antibody escape variants (4, 42, 44, 52). In humans, viral antibody escape variants from the cytopathic influenza virus have been described at the population level (20, 32). For the noncytopathic HIV, antibody escape variants have been isolated from infected individuals (4, 42). Likewise, in H25 transgenic mice, noncytopathic LCMV escaped the neutralizing antibody response in vivo within single individuals. Furthermore, there is accumulating evidence, that neutralizing antibody responses against viruses and bacteria, e.g., vesicular stomatitis virus (31), HIV (6, 15, 19, 24, 33, 41, 59), and Hae-mophilus influenzae (1–3), exhibit very restricted, if not sometimes monoclonal, V-gene usage comparable to the oligoclonal V-gene usage in H25 transgenic mice.

Treatment with the antiviral drug ribavirin as a single agent has been reported to reduce the viral burden of several RNA viruses or at least to diminish clinical symptoms after infection (26, 28, 53, 54). In therapy of human infections, such as Lassa fever and Argentine hemorrhagic fever, all infections with members of the arenavirus family like LCMV, a transient reduction in viral load could be demonstrated (7, 23, 36). Ribavirin is increasingly used in antiviral therapy for hepatitis C but is only effective in a combined treatment with alpha interferon (21, 37, 45, 47). Likewise, in the present study persisting LCMV infection could not be prevented by ribavirin treatment alone. Only the combination with a strong and early virus-neutralizing antibody response efficiently prevented persistent LCMV infection.

The effect of ribavirin on the virus is not absolute and only sterilizing in vitro (23). The main effect of ribavirin in the present study may be to reduce the replication efficiency of LCMV in vivo, thereby rendering the appearance of antibody escape variants considerably less frequent. Although some LCMV antibody escape variants might have been transferred already with the inoculum, the enhanced virus-neutralizing antibodies in ribavirin-treated H25 transgenic mice remained capable of lowering LCMV titer sufficiently so that CTLs were able to control the virus.

In agreement with the present study, virus-specific immune plasma has been shown to exert a beneficial effect of ribavirin on primate survival and control of virus replication after infection with Lassa virus (29). Ribavirin and immune plasma were transferred at the time point of Lassa virus infection. Additive effects were lost if ribavirin and antibodies were transferred later after infection. Possibly, under these circumstances Lassa...
virus had replicated in vivo and generated antibody escape variants randomly before the time point of ribavirin and antibody body transfer. The antibody may have therefore no longer contributed additively to the treatment due to preexistent virus variants.

The present model of antiviral drug treatment in the presence of a strong antiviral antibody response suggests that, if combined, even moderately active antiviral drug treatment plus passive humoral immunotherapy or active vaccination strategies may be efficient against viruses in humans with a tendency to persist, e.g., HBV, HCV, and possibly HIV.

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