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Immunology

Viral cross talk: Intracellular inactivation of the hepatitis B virus during an unrelated viral infection of the liver

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ABSTRACT **Hepatitis B virus (HBV) infection is thought to be controlled by virus-specific cytotoxic T lymphocytes (CTL). We have recently shown that HBV-specific CTL can abolish HBV replication noncytopathically in the liver of transgenic mice by secreting tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) after antigen recognition. We now demonstrate that hepatocellular HBV replication is also abolished noncytopathically during lymphocytic choriomeningitis virus (LCMV) infection, and we show that this process is mediated by TNF-α and IFN-α/β produced by LCMV-infected hepatic macrophages. These results confirm the ability of these inflammatory cytokines to abolish HBV replication; they elucidate the mechanism likely to be responsible for clearance of HBV in chronically infected patients who become superinfected by other hepatotropic viruses; they suggest that pharmacological activation of intrahepatic macrophages may have therapeutic value in chronic HBV infection; and they raise the possibility that conceptually similar events may be operative in other viral infections as well.**

Hepatitis B virus (HBV) is a noncytopathic virus that causes acute and chronic necroinflammatory liver disease and hepatocellular carcinoma. Class I-restricted $CD8^+$ cytotoxic T lymphocytes (CTL) play a key role in the control of HBV infection (reviewed in ref. 1), and it is generally thought that they do so by destroying infected cells. Recently, however, we have shown that adoptively transferred hepatitis B surface antigen (HBsAg)-specific CTL can abolish HBV gene expression (2) and replication (3) in HBV transgenic mice by secreting interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) after antigen recognition. Further, we have reported that these cytokines deliver two noncytopathic antiviral signals to the hepatocyte: one that activates the hepatocyte to degrade HBV RNA posttranscriptionally in a sequence-specific manner (4) and one that causes the hepatocytes to eliminate HBV nucleocapsid particles and their cargo of replicating viral genomes (3). It is possible, therefore, that these noncytopathic antiviral processes may exert an important but previously unsuspected influence on the outcome of HBV infection in man.

Because the effector functions of these antiviral cytokines are independent of their source, HBV gene expression and replication should also be suppressed if the appropriate cytokines are induced in the liver by HBV-nonspecific stimuli. To examine this hypothesis, we monitored HBV gene expression and replication in serum and liver of HBV transgenic mice during lymphocytic choriomeningitis virus (LCMV) infection.

LCMV, an arenavirus that is a natural pathogen of mice, causes a noncytopathic infection of many tissues, including the liver (5). Inoculation of adult immunocompetent mice with moderate doses of most LCMV isolates produces an acute infection, with virus clearance occurring in 7–14 days. LCMV clearance is mediated by $CD8^+$, major histocompatability complex class I-restricted CTL (6). If an effective CTL response is not generated, a persistent infection is established (7). Therefore, mice infected with LCMV at birth or *in utero* develop a lifelong persistent infection because they are unable to mount an effective CTL response, primarily because of thymic deletion of LCMV-reactive T cells (8, 9).

We now report that hepatocellular HBV gene expression and replication are profoundly and noncytopathically suppressed during acute and persistent LCMV infection in HBV transgenic mice, and we demonstrate that these effects are mediated principally by TNF-α and IFN-α/β produced by LCMV-infected intrahepatic macrophages.

MATERIALS AND METHODS

HBV Transgenic Mice. The HBV transgenic mice used in this study (lineage 1.3.32) have been previously described (10). The hepatocytes from these animals replicate HBV from an integrated greater-than-genome-length HBV transcriptional template at levels comparable with that seen in the infected livers of patients with chronic hepatitis, without any evidence of cytopathology (10). Lineage 1.3.32, originally produced in a C57BL/6/SJL hybrid, was expanded by repetitive backcrossing against C57BL/6 and routinely backcrossed one generation against B10.D2 to produce H-2^{bxd} F_1 hybrids before LCMV infection.

LCMV Isolates and Infection of Mice. Two different clones of the WE isolate of LCMV were used in these studies: clones 2.2 and 54 (M. N. Teng, P.B., M.B.A.O., and J. C. de la Torre, unpublished results). The titers of the LCMV stocks, and also infectious virus titers in murine tissues, were determined by plaque assay on Vero cells (11). Adult male HBV transgenic mice (10 weeks old) were infected by i.v. inoculation of 2×10^6 plaque-forming units (pfu) of LCMV WE clone 2.2. Newborn transgenic mice were infected within 24 hr of birth by i.c. inoculation of 10^3 pfu of LCMV WE clone 54.

LCMV-Specific CTL Assay. LCMV-specific CTL activity was quantitated using a standard ^{51}Cr release assay as described by Byrne and Oldstone (12). Effector cells were erythrocyte-depleted splenocyte suspensions from adult mice infected 7 days previously with LCMV WE clone 2.2. Target cells were ^{51}Cr-labeled fibroblast cell lines MC57 (H-2^b), Balb

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Abbreviations: HBV, hepatitis B virus; LCMV, lymphocytic choriomeningitis virus; ALT, alanine aminotransferase; CTL, cytotoxic T lymphocyte; HBsAg, hepatitis B surface antigen; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 2′,5′-OAS, 2′,5′-oligoadenylate synthetase; NP, nucleoprotein; pfu, plaque-forming units; HBcAg, hepatitis B core antigen.

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Cl 7 (H-2^d), and SSSV (H-2^s), and as an allogeneic control, mouse embryo fibroblasts prepared from C3H (H-2^k) mice.

Anti-Cytokine Antibodies. Hamster mAb TN3 19.12 specific for murine TNF-α (250 μg per mouse) (13) and neutralizing sheep Ig to murine IFN-α/β (200 μl per mouse; neutralizing titer: 1×10^6 against 8 international units of murine IFN-α/β) (14) were used in this study. Purified hamster IgG (Jackson ImmunoResearch) and normal sheep Ig (14) were used as control antibodies. One dose of each antibody was administered i.v., first 1 hr before LCMV and then simultaneously with LCMV in all experimental groups of mice.

DNA Analysis. Serum DNase-resistant HBV DNA was measured by dot-blot analysis exactly as described (10). Southern blot analysis was performed on total liver DNA by agarose gel electrophoresis of 20 μg of restricted genomic DNA as described (10).

RNA Analysis. *Northern blot analysis.* Frozen tissues were mechanically pulverized and extracted by the acetic acid/guanidium isothiocyanate/phenol/choloroform method (15). Total RNA (20 μg) was analyzed for HBV and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression by Northern blotting as described (2). Expression of 2′,5′-oligoadenylate synthetase (2′,5′-OAS) was analyzed using a ^{32}P-radiolabeled DNA probe derived from a cDNA clone previously described (16).

RNase protection assay. The RNase protection assay for quantitation of mRNA was performed exactly as described (17). The mouse interleukin (mIL)-1α(B), mIL-1β(A), mIL-

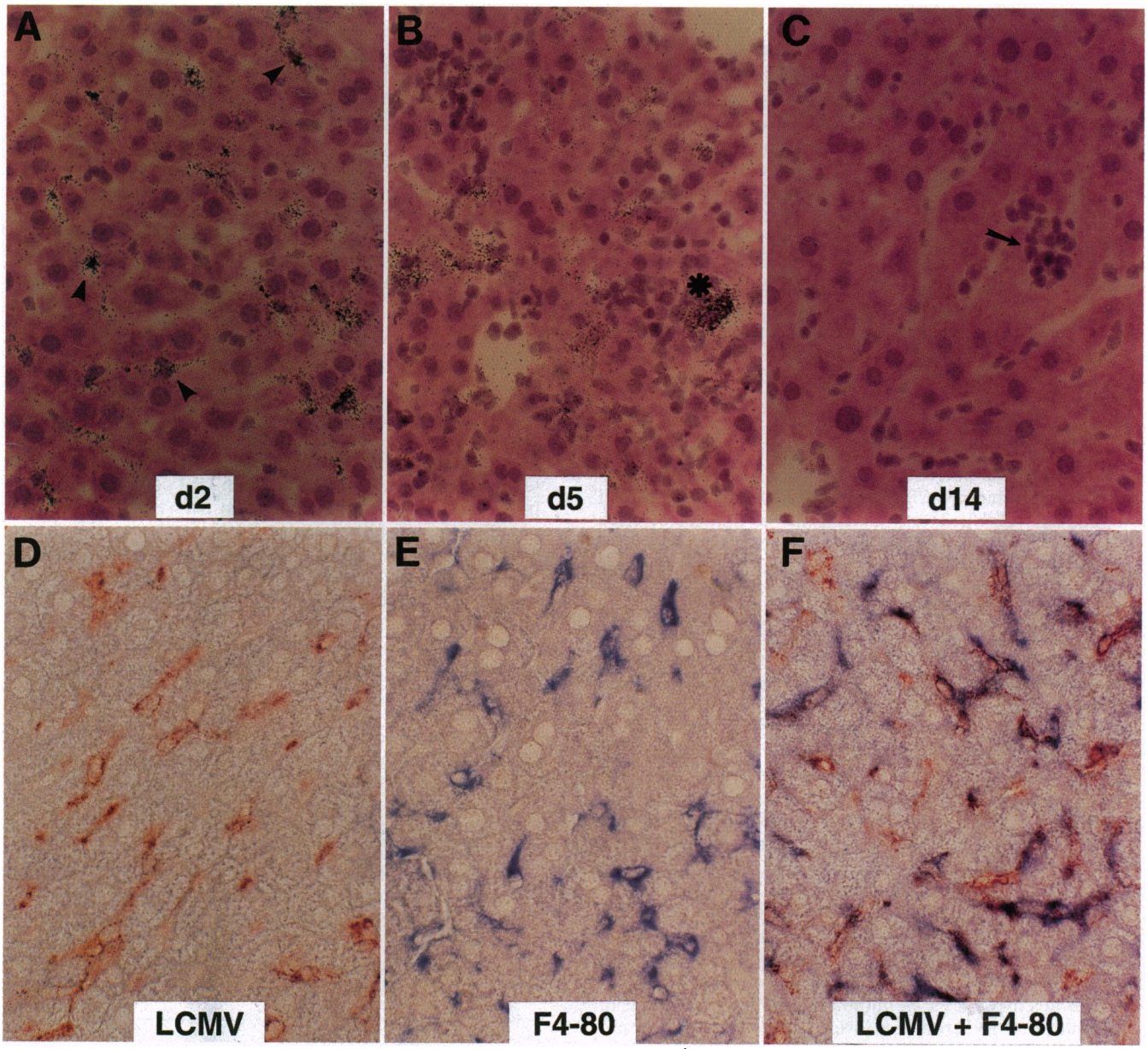

FIG. 1. Transient intrahepatic LCMV infection in HBV transgenic mice. Twenty-eight age-, sex-, and serum HBsAg-matched transgenic mice from lineage 1.3.32 were injected i.v. with LCMV WE, and livers were harvested at various time points thereafter. (*A–C*) Detection of hepatic LCMV mRNAs by *in situ* hybridization of transgenic mice infected 2, 5, and 14 days earlier, respectively. Note that the vast majority of nonparenchymal cells were LCMV RNA-positive at day 2 (*A*, arrowheads) and that less than 5% of hepatocytes were infected at day 5 (*B*, asterisk), at which time many lymphomononuclear cells were present. By day 14 (*C*), LCMV RNA was no longer detectable and the inflammatory infiltrate was profoundly reduced (arrow). (Hematoxylin and eosin; ×120). (*D–F*) Immunohistochemical analysis of liver sections on day 2 after LCMV infection using an LCMV NP-specific mAb (LCMV; *D*), a macrophage-specific mAb (F4–80; *E*), or both (*F*). (Immunoperoxidase stain for LCMV NP and macrophage; ×120).

2(A), mIL-3(B), mIL-4(B), mIL-5(C), mIL-6(B), mouse IFN-γ(B), mouse TNF-α(A), mouse TNF-β(A), and ml32(A) subclones in the pGEM-4 transcription vector were described previously (17). The mCD4(IC), mCD3γ(IC), and mCD8α(DM) subclones in the pGEM-4 vector have been described (3).

In situ hybridization. This procedure was carried out exactly as described (10). The ^{33}P-labeled RNA probe used in this study was prepared by transcription from the T7 promoter of the plasmid pWENP, which was created by cloning the entire LCMV WE nucleoprotein (NP) sequence from a cDNA clone (18) into the plasmid Bluescript KS (Stratagene). pWENP was kindly provided by J. L. Whitton (The Scripps Research Institute, La Jolla, CA).

Biochemical and Histological Analysis of Liver Disease. Hepatocellular injury was monitored by measuring serum alanine aminotransferase (ALT) activity (3). Results were expressed as mean serum ALT activity ± SEM. Tissue samples were fixed in 10% zinc-buffered formalin (Anatech, Battle Creek, MI), embedded in paraffin, sectioned (3 μm), and stained with hematoxylin and eosin as described (3).

Immunohistochemical Analysis. The intracellular distribution of hepatitis B core antigen (HBcAg) was assessed by the labeled avidin-biotin detection procedure exactly as described (3) using rabbit anti-HBc/eAg (Dako) primary antisera. Two-color immunohistochemical staining for LCMV NP and a macrophage-specific antigen was carried out using a mouse IgG2a mAb specific for the LCMV NP (19) and a rat IgG2b mAb (F4–80) to native mouse macrophages (20) based on a method described by Surh and coworkers (21). Fast blue BB (blue) and 3-amino-9-ethylcarbazole (red) were used as coloring substrates for the macrophage-specific antigen and LCMV NP, respectively.

RESULTS

Transient Intrahepatic LCMV Infection in HBV Transgenic Mice. To determine the sites and the extent of LCMV (WE strain) replication in the liver of HBV transgenic mice, 28 serum HBsAg-matched 10-week-old transgenic male mice from lineage 1.3.32 (four mice per group) were i.v. infected with LCMV; groups of four mice were killed at various time points thereafter, and their intrahepatic LCMV RNA distributions were examined by *in situ* hybridization analysis of liver sections using a ^{33}P-labeled LCMV-specific riboprobe.

Two days after inoculation, LCMV RNA was detectable in the vast majority of the nonparenchymal cells in the hepatic sinusoids but not in hepatocytes (Fig. 1). The LCMV signal was detectable as early as 12 hr after infection, increased progressively, and reached maximal intensity in the hepatic nonparenchymal cells 2 days later (Fig. 1*A*, arrowheads). Immunohistochemical analysis revealed that the LCMV-infected nonparenchymal cells were macrophages, presumably Kupffer cells (Fig. 1 *D–F*). Although inflammation was not detectable at this early time point, by 5 days after infection, inflammatory cells were recruited into the liver (Fig. 1*B*), most of the Kupffer cells were still infected, and less than 5% of hepatocytes contained LCMV RNA (Fig. 1*B*, asterisk). By day 14, LCMV RNA was no longer detectable in any cell type, and the inflammatory infiltrate was profoundly reduced (Fig. 1*C*, arrow). The kinetics of LCMV infection observed by *in situ* hybridization were confirmed by determination of viral titers in serum and liver (Fig. 2, lower part).

Suppression of HBV Gene Expression and Replication During Transient LCMV Infection. To examine the effect of transient LCMV infection on hepatic HBV gene expression and replication, Northern and Southern blot analyses were performed on total hepatic RNA and DNA, respectively, from the mice described in Fig. 1. The results were compared with total hepatic RNA and DNA pooled from 10 age-, sex-, and serum HBsAg-matched uninjected transgenic controls.

As shown in Fig. 2 for two representative mice per time point, the hepatic steady-state content of 3.5- and 2.1-kb HBV mRNA decreased 3- to 5-fold (based on densitometric analysis; not shown) on 2 days after LCMV infection, almost disappeared in some animals between days 5 and 14, and returned to baseline levels 4 weeks after infection. More important, as shown in Fig. 2, HBV DNA replicative forms

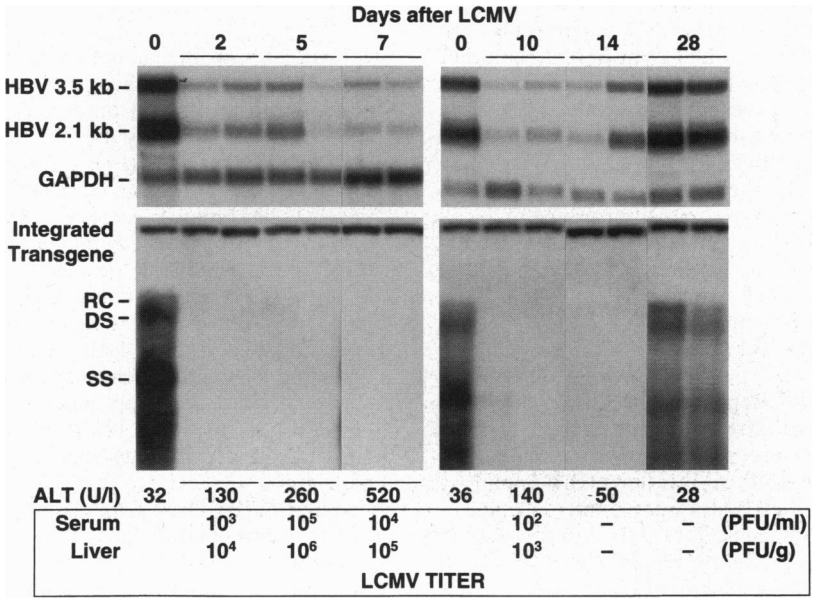

FIG. 2. Suppression of HBV gene expression and replication during transient LCMV infection in transgenic mice from lineage 1.3.32. Time course. Groups of four mice (see Fig. 1) were killed after infection with LCMV. (*Top*) The steady-state HBV and GAPDH mRNA contents were compared with total RNA pooled from 10 age-, sex-, and serum HBsAg-matched uninfected (time 0) controls. (*Middle*) HBV DNA content was compared with total DNA pooled from the same controls by hybridization with a ^{32}P-labeled HBV-specific DNA probe. DNA loading was normalized by comparing the signal intensity of the integrated transgene DNA seen at the top of each lane. Bands corresponding to the integrated transgene are indicated as follows: relaxed circular (RC), double-stranded linear (DS), and single-stranded (SS) HBV DNAs. (*Bottom*) The mean serum ALT activity and serum and liver LCMV titers are expressed as units/liter, pfu/ml, and pfu/g, respectively.

disappeared from the livers by 2 days after LCMV infection (and even by 12 hr, see below) and remained undetectable until reappearing at the day 28 time point, 2 weeks after clearance of LCMV. HBV replication occurs within HBV nucleocapsid particles in these animals (10) as well as during natural infection (22), and these nucleocapsids are detectable immunohistochemically in the cytoplasm of the hepatocytes as HBcAg (10). In the mice, cytoplasmic HBcAg immunoreactivity and replicative intermediates are present in approximately one-third of the hepatocytes, all of which are located in the center of the hepatic lobule (10). After LCMV infection, cytoplasmic HBcAg disappeared from all of these hepatocytes with the same kinetics as the replicative intermediates (not shown). Not surprisingly, the disappearance of HBV DNA replicative intermediates was accompanied by the disappearance of circulating virions, measured as HBV DNA in pooled serum from groups of four mice at each time point (not shown). It is noteworthy that serum ALT activity was only slightly elevated throughout the entire infection (Fig. 2, *Bottom*), especially at day 2, when HBV replication was virtually abolished, indicating that the loss of HBV replicative forms was not due to the destruction of hepatocytes. By comparing the serum ALT profile in these mice with known standard profiles (3), we estimated that <5% of hepatocytes were destroyed during acute LCMV infection. This is compatible with the observation that the frequency of LCMV-positive hepatocytes detected at the peak of infection (Fig. 1*B*) is much lower than the frequency of hepatocytes that replicate the HBV genome in these mice (10), further confirming that suppression of HBV replication by LCMV is not due to the destruction of hepatocytes.

Intrahepatic Cytokine Gene Expression During Transient LCMV Infection in HBV Transgenic Mice. To analyze the intrahepatic inflammatory response in these animals, we monitored the hepatic content of mRNA for CD3γ, CD4, CD8α, various cytokines, and 2′,5′-OAS (a marker of IFN-α/β induction) in the same transgenic livers at multiple time points after LCMV infection. Low levels of the monokines IL-1α and IL-1β were the only mRNA species detected in livers of uninjected controls (Fig. 3*A*), and all T-cell markers were absent (Fig. 3*B*). Two days after infection, TNF-α and 2′,5′-OAS were induced (Fig. 3) and LCMV RNA and proteins were detected in Kupffer cells (Fig. 1 *A* and *D*), whereas the other cytokines and housekeeping gene products (L32 and GAPDH) that were used as internal controls were basically unchanged (Fig. 3). Importantly, all of the T-cell markers were negative at this time point (Fig. 3*B*). By 5 days after infection, TNF-α and IL-1α and -1β expression increased further, concomitant with the induction of IFN-γ (Fig. 3*A*) and the appearance of T-cell markers (Fig. 3*B*), all of which remained elevated for at least 14 days. These later patterns probably reflected the induction of an LCMV-specific $CD8^+$ CTL response in the liver and the recruitment of activated inflammatory cells, because LCMV-specific CTL were detected in the spleen 7 days after infection (not shown).

Early Suppression of HBV Replication by LCMV Is Mediated by IFN-α/β and TNF-α. To determine the extent to which type I IFNs and TNF-α were responsible for the earliest LCMV-induced effects before the infiltration of inflammatory cells in the acutely infected HBV transgenic mice, we monitored the ability of mAbs specific for TNF-α and sheep Ig against murine IFN-α/β to modulate LCMV titer, 2′,5′-OAS mRNA induction, and HBV replication 12 hr after LCMV infection. Groups of mice were injected i.v. with sheep antiserum against murine IFN-α/β, or mAb against murine TNF-α, or both antibodies together, 1 hr before and simultaneously with the i.v. injection of 2×10^6 pfu of LCMV. Control mice were injected with irrelevant hamster IgG and normal sheep Ig. HBV replication was virtually abolished in the LCMV-infected antibody control mice 12 hr after infection,

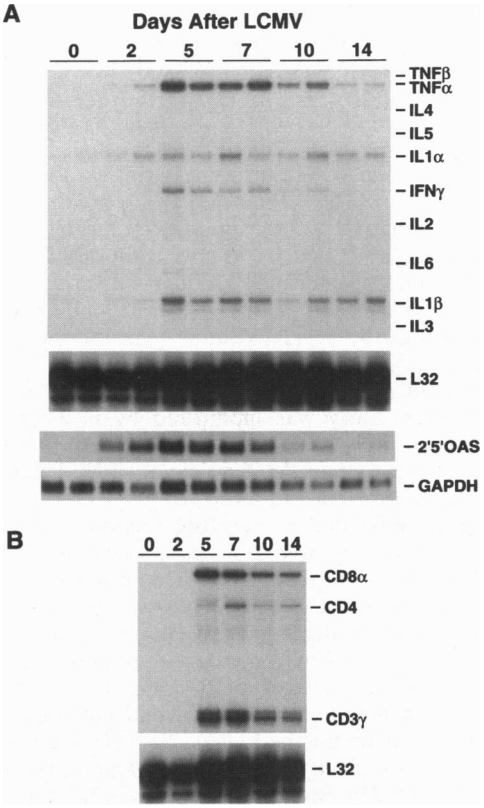

FIG. 3. Analysis of intrahepatic T-cell markers and cytokine mRNA expression. (*A*) Total RNA extracted from the mice described in the legend to Fig. 2 was analyzed by RNase protection for the expression of assorted cytokine transcripts. Total RNA was also analyzed by Northern blotting for the expression of 2′,5′-OAS. (*B*). Total RNA was analyzed by RNase protection for the expression of CD4, CD8α, and CD3γ. The two lanes on the left of each gel represent nontransgenic and transgenic resting livers, respectively. The mRNAs encoding the ribosomal protein L32 and the housekeeping enzyme GAPDH were used to normalize the amount of RNA loaded in each lane.

coincident with the induction of 2′,5′-OAS (Fig. 4). Importantly, both of these changes were blocked by simultaneous administration of both antibodies, even though the LCMV titer was ≈10 times higher in the serum and liver in the antibody-treated mice (Fig. 4). Notably, the ALT activity was minimally elevated to an equivalent degree in both groups of mice at this time, irrespective of the degree to which HBV replication was reduced, indicating again that this antiviral effect is not due to the destruction of hepatocytes.

These results indicate that IFN-α/β and TNF-α are principally responsible, either directly or indirectly, for the early, noncytolytic control of HBV replication in this model. When the same antibodies were administered separately, however, the LCMV-induced suppression of HBV replication was only partially blocked at the 12-hr time point (not shown), suggesting that these cytokines may activate independent antiviral pathways.

Hepatic HBV Gene Expression and Replication Are Permanently Suppressed in HBV Transgenic Mice Persistently Infected with LCMV. To determine whether HBV gene expression and replication are modulated during persistent LCMV infection, four newborn HBV transgenic mice from lineage 1.3.32 were infected intracranially with LCMV and killed 8 weeks later. LCMV titer, serum ALT activity, and the intrahepatic expression of cytokines, HBV RNA, HBV DNA, and HBcAg were measured at the time of autopsy. As shown in Fig. 5*A*, HBV DNA replicative forms were virtually abolished in the persistently infected livers, and the 3.5- and 2.1-kb HBV

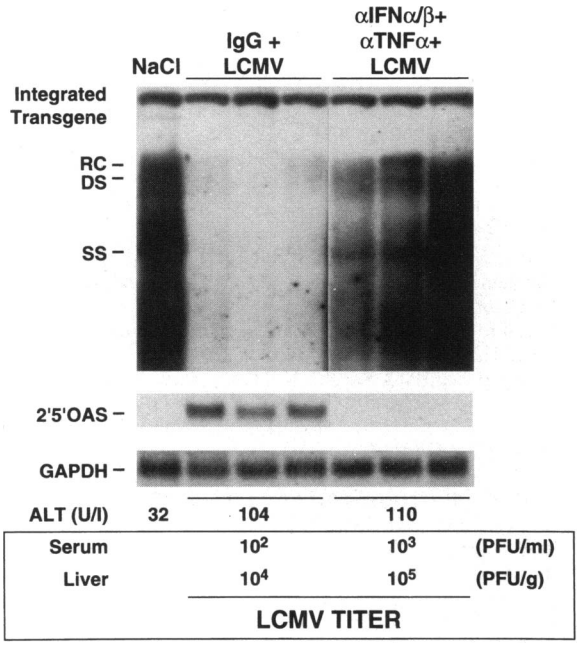

FIG. 4. IFN-α/β and TNF-α mediate the early suppression of HBV replication by LCMV. (*Top*) Southern blot analysis of total liver DNA isolated from groups of three age-, sex-, and serum HBsAg-matched transgenic mice that were i.v. injected with a combination of antibodies to IFN-α/β and TNF-α or with control antibodies 1 hr before LCMV and simultaneously with LCMV and were killed 12 hr later. Control mice were injected with NaCl. (*Middle*) The steady-state 2′,5′-OAS and GAPDH mRNA content was compared by Northern blot analysis with total RNA pooled from NaCl-injected controls. (*Bottom*) The mean serum ALT activity and serum and liver LCMV titers are expressed as units/liter, pfu/ml, and pfu/g, respectively.

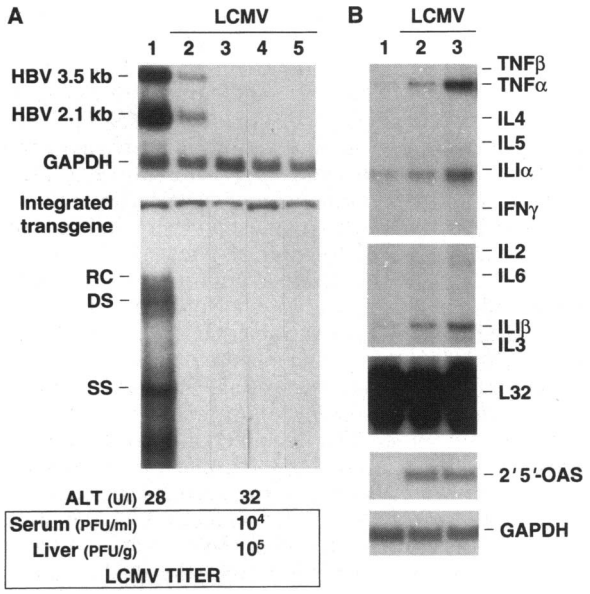

FIG. 5. Hepatic HBV gene expression and replication are permanently suppressed in HBV transgenic mice persistently infected with LCMV. (*A*, *Top*) Northern and Southern blot analyses were performed on total hepatic RNA and DNA extracted from four transgenic males (lanes 2–5) that were infected within 24 hr of birth by intracranial inoculation of 1×10^3 pfu of LCMV WE clone 54 and killed 8 weeks later. The steady-state HBV and GAPDH mRNA and HBV DNA contents were compared with total RNA and DNA pooled from 10 age-, sex-, and serum HBsAg-matched uninfected controls (lane 1). (*A*, *Bottom*) The mean serum ALT activity and serum and liver LCMV titers are expressed as units/liter, pfu/ml, and pfu/g, respectively. (*B*, *Top*). Analysis of intrahepatic cytokine mRNA expression in two HBV transgenic animals persistently infected with LCMV (lanes 2 and 3) and in total RNA pooled from the same controls (lane 1). The ribosomal protein L32 was used to normalize the amount of RNA loaded in each lane. (*B*, *Bottom*) The expression of 2′,5′-OAS was analyzed by Northern blotting in the same livers as indicated. mRNA encoding the housekeeping enzyme GAPDH was used to normalize the amount of RNA loaded in each lane.

RNA contents of the livers were profoundly reduced. Furthermore, all traces of hepatic HBcAg had vanished from the livers of these animals (not shown), as compared with uninfected, age- and sex-matched HBV transgenic mice (not shown). The disappearance of all of these HBV gene products from the persistently LCMV-infected livers occurred in the absence of any increase in serum ALT activity (Fig. 5*A*) or histological evidence of liver cell injury, inflammation, or regeneration (not shown), indicating again that LCMV-induced suppression of HBV replication is not due to the destruction of hepatocytes. Importantly, intrahepatic TNF-α and 2′,5′-OAS were induced (Fig. 5*B*), coincident with massive LCMV infection of virtually all intrahepatic macrophages and 5–10% of hepatocytes, as determined by *in situ* hybridization analysis (not shown), strongly suggesting that TNF-α and type I interferons contribute to suppression of HBV replication during persistent LCMV infection as they do during the first 12 hr of acute LCMV infection (see above).

DISCUSSION

The current study was performed to determine whether inflammatory cytokines produced during a noncytopathic virus infection of the liver could suppress the replication of an unrelated virus, specifically HBV. LCMV was chosen for these experiments because, like HBV, it is noncytopathic and it is controlled by a class I-restricted, $CD8^+$ CTL response (12) that, in infected adult mice, causes an acute necroinflammatory liver disease resembling human acute viral hepatitis (23). We selected a hepatotropic strain of LCMV (WE) (11) and inoculation conditions known to induce a mild transient necroinflammatory liver disease in adult animals and a persistent noninflammatory hepatic infection in neonatally injected animals in the context of the $H\text{-}2^{dxb}$ major histocompatability complex genotype (23).

The results of this study demonstrate that hepatocellular HBV replication can be abolished by noncytopathic, cytokine-dependent pathways activated by an unrelated viral infection of the liver, even when that infection is principally limited to a different cell type, in this case the macrophage. This suggests that the induction of cytokine activity within the liver by agents (viral or otherwise) that activate resident macrophages or other cell types to produce these cytokines may have therapeutic value in chronic HBV infection.

The data may also explain the known suppressive effect of hepatitis delta virus on woodchuck hepatitis virus replication in chronically infected woodchucks (24, 25) and the anecdotal clinical reports of HBV clearance in chronically infected patients during supervening acute hepatitis A (26), hepatitis C (27), or hepatitis delta (28) virus infections.

The current observations also underscore the importance of inflammatory cytokines in the control of other viral infections besides HBV. For example, a variety of cellular antiviral proteins are induced in response to IFN activation, some of which have been shown to impair influenza and vesicular stomatitis virus transcription (29, 30), whereas others lead to cleavage of single-stranded picornaviral RNA (31). Additionally, TNF-α and/or TNF-β have been reported to inhibit the replication of herpes simplex virus, adenovirus, and vesicular stomatitis virus (32), and HIV transcription has been shown to be inhibited by currently undefined noncytolytic factors produced by HIV-specific class I-restricted CTL upon antigen

recognition (33). Interestingly, a number of viruses encode proteins that block the transcriptional activation of the IFN-activatable genes (34, 35) or represent receptor analogues for IFN-α/β, IFN-γ, and TNF-α (36–38), apparently as a strategy to blunt the antiviral activity of these cytokines.

Although the foregoing observations indicate that curative properties of certain inflammatory cytokines may play an important role in the outcome of many viral infections, including HBV (3), clearance of most noncytopathic infections also depends on the destruction of infected cells by virus-specific CTL (39). The relative importance of these two curative and destructive pathways in the clearance of most noncytopathic viruses is not known at this time. In our opinion, however, viral clearance may depend more heavily on noncytopathic curative mechanisms, especially in massive infections of vital organs, because the host must preserve as much tissue as possible while eliminating the infecting virus. Furthermore, curative mechanisms are likely to be much more efficient at viral clearance than cytodestructive mechanisms, because the cytokines are readily diffusible, whereas CTL killing is a relatively inefficient one-on-one process.

If the foregoing hypothesis is correct, this dual destructive/curative process could influence the host–virus relationship in several ways. First, it would provide the selective pressure needed to explain the coevolution of cytopathic and noncytopathic antiviral functions in the effector limb of the immune response. Second, it could influence the mutational threshold required for cytokine-sensitive viruses to escape immunological control. Finally, it could explain the spectrum of outcomes observed during viral infection in different individuals, with dominance of the curative process leading to viral clearance and recovery and dominance of the destructive pathway leading either to low-grade chronic inflammation and viral persistence or to massive tissue destruction and the death of the host.

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