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Interleukin-2 and Alpha/Beta Interferon Down-Regulate Hepatitis B Virus Gene Expression In Vivo by Tumor Necrosis Factor-Dependent and -Independent Pathways†

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We have recently reported that administration of recombinant tumor necrosis factor alpha (TNF-α) to hepatitis B virus (HBV) transgenic mice reduces the hepatic steady-state content of HBV-specific mRNA by up to 80% in the absence of liver cell injury. In the current study, we analyzed the regulatory effects of several other inflammatory cytokines in the same transgenic model system. Hepatic HBV mRNA content was reduced by up to 90% following administration of a single noncytopathic dose (100,000 U) of interleukin 2 (IL-2). Comparable effects were produced by administration of alpha and beta interferons (IFN-α and IFN-β), but only after multiple injections of at least 500,000 U per mouse. Importantly, the regulatory effect of IL-2 was completely blocked by the prior administration of antibodies to tumor necrosis factor alpha (TNF-α), which did not block the effect of IFN-α or IFN-β. In contrast to these observations, recombinant IFN-γ, IL-1, IL-3, IL-6, TNF-β, transforming growth factor beta, and granulocyte-monocyte colony-stimulating factor were inactive in this system. These results suggest that selected inflammatory cytokines can down-regulate HBV gene expression in vivo by at least two pathways, one that is dependent on TNF-α and another that is not. These results imply that antigen-nonspecific products of the intrahepatic HBV-specific inflammatory response may contribute to viral clearance or persistence during HBV infection.

The cellular immune response to hepatitis B virus (HBV)-encoded antigens is thought to play an important role in viral clearance and disease pathogenesis during acute and chronic HBV infection. In keeping with this notion, we and others have reported that HLA class I- and class II-restricted HBV-specific T cells are readily detectable in the peripheral blood of patients with acute hepatitis who successfully clear the virus, but that they are much less abundant in persistently infected patients with chronic hepatitis (4, 27, 30). In chronically infected patients, however, class I- and class II-restricted HBV-specific T cells have been isolated from the hepatic parenchyma, where it is assumed that they contribute to the destruction of some, but not all, of the infected hepatocytes, thereby permitting the virus to persist, while contributing to the pathogenesis of chronic liver disease (28). However, recent evidence suggests that this hypothesis may be too simplistic and that the immunological processes that lead to liver cell injury, viral clearance, and viral persistence may be more complex than is currently thought.

For example, we have recently reported that class I-restricted HBV surface antigen (HBsAg)-specific cytotoxic T-lymphocyte (CTL) clones cause an acute necroinflammatory liver disease when they are transferred to HBsAg transgenic mice, but that an important component of this disease process is mediated by inflammatory cytokines secreted by the CTL, especially gamma interferon (IFN-γ) (1, 2). We have also shown that under noncytopathic conditions, the same CTL can down-regulate hepatic HBV mRNA content in these animals and that this effect is mediated by their ability to secrete and/or to induce IFN-γ and tumor necrosis factor alpha (TNF-α) (16a). Furthermore, we have shown that the regulatory effect of TNF-α can be reproduced by the administration of noncytotoxic doses of recombinant TNF-α to these animals (14) and that this effect is mediated at a posttranscriptional level in vivo (17).

Accordingly, we have become interested in the possibility that selected inflammatory cytokines, potentially derived from the HBV-specific T cells and the inflammatory cells that they recruit, might contribute to viral clearance and/or disease pathogenesis by modulating hepatocellular HBV gene expression during naturally acquired infection. In this study, therefore, we extended our analysis to a large panel of additional cytokines.

We now report that HBV gene expression is profoundly down-regulated following administration of a single noncytopathic dose of recombinant interleukin-2 (IL-2) and after multiple high doses of recombinant IFN-α and IFN-β, but not by any of the other cytokines that we tested, including IFN-γ. Interestingly, we found that the regulatory effect of IL-2 is mediated by TNF-α while that of IFN-α or IFN-β is not, indicating the existence of at least two independent pathways for the control of HBV gene expression by inflammatory cytokines in vivo.

MATERIALS AND METHODS

Transgenic mice. The HBV transgenic lineage pFC80-219 used in these studies was produced by microinjection of a 12.9-kb *Hin*dIII-*Pst*I fragment excised from plasmid pFC80 that contains four complete HBV genomes (ayw subtype) linked in a tandem head-to-tail orientation at the HBV *Eco*RI site of pBR322 (6). Structural analysis of the transgene reveals that it is integrated at a single site in the mouse genome and that at least one complete, uninterrupted HBV genome is present. The sera of these mice contain 4 to 8 μg/ml of HBsAg

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(Ausria II; Abbott Laboratories, North Chicago, Ill.) per ml, which was used as a convenient marker to identify transgenic animals and to normalize experimental and control groups for transgene expression in each experiment. In all experiments, mice were anesthetized with Metophane (Pitman-Moore, Mundelein, Ill.) prior to phlebotomy from the retro-orbital plexus or before they were sacrificed by cervical dislocation.

RNA isolation and Northern (RNA) blot analysis. The left hepatic lobe was immediately removed, snap frozen in liquid nitrogen, and stored at −70°C. Frozen tissue was mechanically pulverized, and total hepatic RNA was prepared by the acid guanidinium-phenol-chloroform method (7). RNA (20 μg) was electrophoretically fractionated in 1% formaldehyde agarose gels, blotted by capillary transfer, and transferred to nylon membranes (Hybond-N; Amersham, Arlington Heights, Ill.). Membranes were prehybridized at 42°C for 4 h in 50% formamide–5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–2× Denhardt's solution–0.1% sodium dodecyl sulfate (SDS)–200 μg of denatured salmon sperm DNA per ml and hybridized in the same solution plus 10% dextran sulfate for 18 to 24 h at 42°C. The nylon filters were hybridized to ^{32}P-radiolabeled DNA probes of specific activities approaching 10^9 dpm/μg that were synthesized from random oligonucleotide-primed plasmids containing the cloned sequences of the entire HBV genome (pFC80) (6), the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (12), and the metallothionein I gene (33). Membranes were washed three times for 1 h each time in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS, 0.5× SSC–0.1% SDS, and 0.1× SSC–0.1% SDS, respectively, at 68°C according to the standard protocol and exposed onto X-ray film (Kodak, Rochester, N.Y.). Northern blot analysis (below) reveals that the only HBV-derived mRNA in lineage 80-219 is a 2.1-kb species corresponding to the expected size of the transcript originating from the pre-S2 promoter. No significant variation in baseline expression of hepatic HBV steady-state mRNA was detected among heterozygous age-, sex-, and serum HBsAg-matched transgenic offspring.

Analysis of hepatocellular injury. Hepatocellular injury was assessed by the measurement of alanine aminotransferase activity in serum, using a Hitachi 705 autoanalyzer. Tissue samples were fixed in 10% zinc-buffered formalin (Anatek, Ltd., Battle Creek, Mich.), embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin.

Cytokines and antibodies. Recombinant murine TNF-α (lot 4296-17; specific activity, 1.2×10^6 U/mg; 0.511 endotoxin units [EU]/ml *Limulus* amebocyte lysate assay [LAL]), recombinant murine IFN-γ (lot M3-RD48; specific activity, 5.2×10^6 U/ml; 1.1 mg/ml; <10 EU/ml [LAL]), recombinant human TNF-β (lot 4296-18; specific activity, 2.1×10^8 U/mg; 3.13 EU/mg [LAL]), and recombinant human transforming growth factor beta (TGF-β) (lot G098AD; 0.1 mg/ml; <0.5 EU/ml [LAL]) were generously provided by S. Kramer (Genentech, Inc., South San Francisco, Calif.). Recombinant human IL-6 (batch PPG9001; specific activity, 52×10^6 U/mg; 2.5 mg/ml; <0.4 EU/ml [LAL]) was kindly provided by Ekke Liehl (Sandoz Forschungstitut, Vienna, Austria). Recombinant human IFN-αA/D (lot 102AA; specific activity, 6.4×10^7 U/mg [L929 cell-vesicular stomatitis virus [VSV] assay]) was generously provided by P. F. Sorter (Hoffmann-La Roche, Nutley, N.J.). Recombinant human IL-1α (lot 2/88; specific activity, 3×10^8 U/mg; 0.68 mg/ml; 0.5 EU/ml [LAL]) was a generous gift from Peter Lomedico (Hoffmann-La Roche). Recombinant murine IFN-β (lot M0034; specific activity, 10^7 U/ml; no detectable endotoxin) was kindly provided by M. Moriyama (Toray Industries, Inc., Tokyo, Japan). Recombinant murine IL-2 (lot. 3353-023; specific activity, 5×10^7 U/mg; 100 μg/ml; <1 EU/ml [LAL]) and recombinant murine IL-3 (specific activity, 5×10^5 U/mg; 100 μg/ml) were generously provided by S. Gillis (Immunex, Seattle, Wash.). A hamster monoclonal antibody (MAb) with specificity for murine TNF-α (34) (5.05 mg/ml) was a generous gift from R. D. Schreiber (Washington University School of Medicine, St. Louis, Mo.). Cytokines were diluted in 0.8% NaCl solution (saline) (endotoxin, <0.25 EU/ml [LAL]) prior intraperitoneal (i.p.) injection as previously described (14).

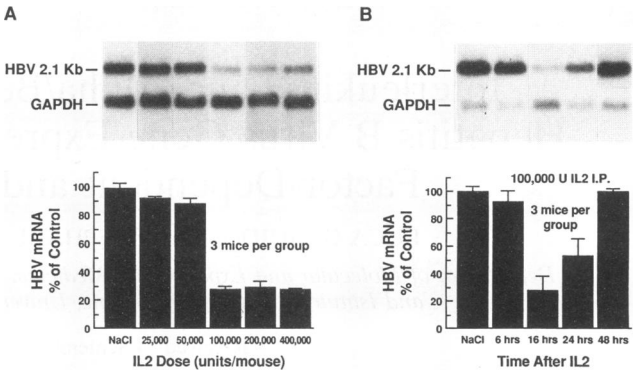

FIG. 1. Effect of IL-2 on hepatic HBV mRNA content. (A) In vivo dose response. Shown is Northern blot analysis of 20 μg of total hepatic RNA isolated 16 h after a single i.p. injection of 25,000, 50,000, 100,000, 200,000, or 400,000 U of recombinant murine IL-2 into groups of three lineage 80-219 littermates, matched for age, sex, and serum HBsAg. Results are compared with the hepatic HBV mRNA content of a pool from 10 transgenic mice injected with 0.1 N NaCl. (B) In vivo kinetics. Shown is Northern blot analysis of 20 μg of total liver RNA isolated 6, 16, 24, or 48 h after groups of three transgenic mice received a single i.p. injection of 100,000 U of recombinant murine IL-2. Saline control RNA was prepared as described in the text. The housekeeping GAPDH gene transcript was used to normalize the amount of RNA bound to the membrane. Bars represent mean ± 1 standard deviation of densitometric analysis, calculated as HBV/GAPDH ratio.

RESULTS

Effect of IL-2 on hepatic HBV mRNA content. (i) In vivo dose response. Six groups of age-, sex-, and serum HBsAg-matched transgenic littermates were injected i.p. either with saline (10 animals) or with 25,000, 50,000, 100,000, 200,000, and 400,000 U of IL-2 (3 animals per group), and livers were harvested for Northern analysis 16 h later. Total RNA pooled from the saline-treated mice was used as a control. As shown in Fig. 1A, densitometric analysis of Northern autoradiographs indicated that the hepatic steady-state content of HBV mRNA was reduced by 70 to 80% at IL-2 doses of 100,000 U or more per mouse. No sign of hepatocellular injury was detected by histological analysis in any of these animals, even at the highest dose level (not shown), or by measurement of alanine aminotransferase activity (not shown).

(ii) In vivo kinetics. On the basis of these observations, 12 age-, sex-, and serum HBsAg-matched transgenic littermates were each injected i.p. with 100,000 U of IL-2, and the livers from groups of three mice were harvested 6, 16, 24, and 48 h later. As shown in Fig. 1B, the hepatic 2.1-kb HBV mRNA content was unchanged 6 h after IL-2 administration, was maximally reduced at the 16-h sampling interval, and returned to the normal level between 24 and 48 h later.

TNF-α mediates the regulatory effect of IL-2. Since hepatocytes are not known to express IL-2 receptors (35), the IL-2

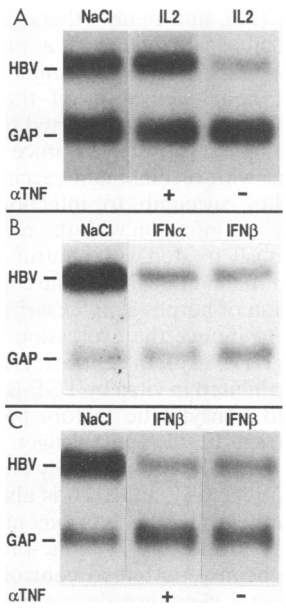

FIG. 2. IL-2, IFN-α, and IFN-β inhibit HBV gene expression by TNF-α-dependent and -independent pathways. (A) Two groups of four transgenic mice were injected i.p. either with 250 μg of a hamster MAb specific for murine TNF-α (+) or with nonspecific hamster IgG (−) 24 h before the administration of 200,000 Units of IL-2, and the mice were killed 16 h later. (B) Groups of three transgenic mice were injected i.p. once daily for 6 days with 500,000 U of IFN-α or IFN-β, and the mice were killed 16 h after the last injection. (C) Transgenic mice that received daily injections of IFN-β (500,000 U i.p.) for 6 days were injected i.p. either with 250 μg of a hamster MAb specific for murine TNF-α (+) or with nonspecific hamster IgG (−). The antibodies were injected 24 h and 3 days after the first IFN-β injection, and the mice were killed 16 h after the last IFN-β injection. Twenty micrograms of total liver RNA was analyzed for HBV and GAPDH (GAP) expression by Northern blotting as described in the legend to Fig. 1. Representative samples from each group are shown. Hepatic RNA pooled from 10 saline-injected transgenic mice is included as a control in all three panels.

effect on hepatocellular HBV mRNA content must be indirect. Since IL-2 receptors are present on intrahepatic macrophages (8, 9), and since we have previously demonstrated that TNF-α (a macrophage product) also causes a profound reduction in hepatic HBV mRNA content in this lineage, we examined the possibility that the IL-2 effect was mediated by TNF-α. In this study, groups of four mice were injected i.p. either with 250 μg of a hamster MAb specific for murine TNF-α or with nonspecific hamster immunoglobulin G (IgG) 24 h before the administration of 200,000 U of IL-2. The animals were sacrificed 16 h later, and the livers were processed for hepatic HBV and GAPDH mRNA content. As illustrated in Fig. 2, the regulatory effect of IL-2 was virtually completely abolished by the prior administration of anti-TNF-α MAb, while the control hamster IgG had no effect.

Effects of IFN-α and IFN-β on hepatic HBV mRNA content. We previously reported (14) that a single injection of 500,000 U of IFN-α caused a minor reduction of hepatic HBV-specific mRNA content in vivo. To confirm and extend this observation, groups of three mice were injected i.p. once daily for 6 days with 500,000 U of IFN-α or IFN-β, and their livers were analyzed for HBV mRNA content 16 h after the last injection. As shown in Fig. 2, both of these cytokines suppressed hepatic HBV mRNA content by 70 to 80%, in the absence of hepatocellular injury (not shown), when they were administered daily at this high dosage level for several days. Multiple daily injections of a lower dose (100,000 U) and single injections of a higher dose (1,000,000 U) of IFN-β were less effective, reducing the HBV mRNA content by less than 50% at the 16-h time point (not shown).

TNF-α does not mediate the regulatory effect of IFN-β. To determine whether the regulatory effect of the IFNs was mediated by TNF-α, we injected 250 μg of anti-TNF-α MAb or control hamster IgG to two groups of three mice that received daily injections of IFN-β (500,000 U i.p.) for 6 days. The antibodies were injected 24 h before and 3 days after the first IFN-β injection. At 16 h after the last injection, total liver RNA was analyzed as described above. As shown in Fig. 2, the regulatory effect of IFN-β was not affected by anti-TNF-α MAb. Since anti-TNF-α MAb has been shown to circulate with a half-life of 7 days after i.p. administration in mice (34), the anti-TNF-α MAb levels were probably sufficient to neutralize any TNF-α that might have been induced by the repetitive administration of IFN-β. We conclude from these experiments that IFN-α and IFN-β modulate HBV gene expression by a pathway that is TNF-α independent and therefore different from the pathway responsible for the effect of IL-2.

Effects of cytokine combinations on hepatic HBV mRNA content. To assess the possibility that the regulatory cytokines function in an additive or synergistic manner to influence hepatic HBV mRNA content, groups of four mice were injected i.p. with IL-2 (100,000 U), TNF-α (80,000 U), IFN-α (500,000 U), or IFN-β (500,000 U) separately or in combination. Mice receiving IL-2 and TNF-α, either alone or in combination, were killed 16 h after injection. Mice receiving IFN-β were injected daily for 6 days either alone or together (last dose only) with IL-2, TNF-α, or both. Sixteen hours later, livers were harvested for analysis of HBV mRNA content. As shown in Fig. 3, the combined administration of IL-2 plus TNF-α or IL-2 plus IFN-β led to only a slight enhancement of the regulatory effect of each cytokine given alone. In contrast, the combination of TNF-α plus IFN-β appeared to be significantly more effective than either cytokine alone, and the regulatory effect may have been even further enhanced by inclusion of IL-2 in the treatment regimen, although we have never observed 100% reduction of HBV mRNA content in these experiments. It is interesting that the reduction in HBV gene expression was not accompanied by a strong hepatic acute-phase response, measured here by metallothionein gene expression, except when TNF-α was administered, indicating that the regulation of HBV and other cellular genes is not necessarily linked in this model.

Hepatic HBV mRNA content is unaffected by the administration of other recombinant cytokines. In contrast to the foregoing observations, we did not observe any change in hepatic HBV mRNA content in groups of three mice injected with IL-1, IL-6, TGF-β, TNF-β, granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-3 at multiple dosage levels and injection schedules (Table 1). Importantly, we also failed to observe any effect following the administration of recombinant murine IFN-γ in response to either single or multiple (six) daily injections of either 100,000 or 500,000 U of the cytokine, in contrast to the effects of IFN-α and IFN-β.

DISCUSSION

The inflammatory cytokines constitute a large group of pleiotropic proteins that exert complex hormone-like, autocrine, and/or paracrine functions after binding to specific

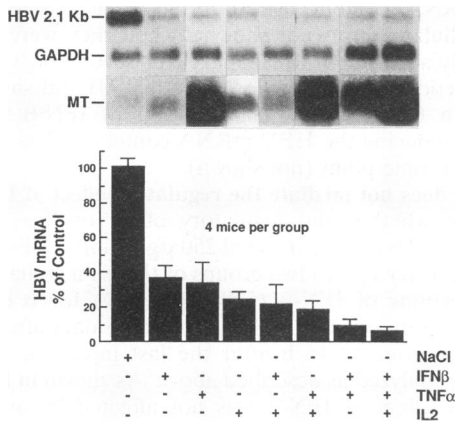

FIG. 3. Effect of cytokine combinations on hepatic HBV mRNA content. Groups of four mice were injected i.p. with IL-2 (100,000 U), TNF-α (80,000 U), IFN-α (500,000 U), or IFN-β (500,000 U) separately or in combination. Mice receiving IL-2 and TNF-α, either alone or in combination, were killed 16 h after injection. Mice receiving IFN-β were injected daily for 6 days either alone or together (last dose only) with IL-2, TNF-α, or both. Sixteen hours later, livers were harvested for analysis of HBV mRNA content. Filters were hybridized with HBV-, GAPDH-, and metallothionein I (MT)-specific probes as previously described. Bars represent mean ± 1 standard deviation of densitometric analysis calculated as HBV/GAPDH ratio. Results were compared with the hepatic HBV and GAPDH mRNA content of a group of 10 saline-injected control mice.

high-affinity receptors on their target cells. The antiviral potential of certain cytokines, particularly the IFNs, has been known for many years (20). A variety of cellular antiviral proteins are up-regulated in response to IFN activation; these proteins include Mx protein, a potent inhibitor of influenza virus and VSV replication that appears to impair viral transcription (19, 37), 2′-5′-oligoadenylate synthetase and RNase L, which cooperatively lead to cleavage of single-stranded picornavirus RNA (23), and many others (36). Interestingly, apparently as a defense mechanism, a number of viruses encode proteins, such as the HBV terminal protein (3), that block the transcriptional activation of the IFN-activatable genes (13, 21). IL-2 has also been reported to display antiviral properties. For example, athymic nude mice have been shown to resolve infections with recombinant vaccinia viruses encoding IL-2, while they succumb to infection with wild-type vaccinia virus as well as infection with the recombinant virus if they are simultaneously treated with neutralizing antibodies to IL-2 (22, 32). TNF-α and/or TNF-β have been reported to inhibit the replication of herpes simplex virus, adenovirus, and VSV (26, 41) and to induce the expression of human immunodeficiency virus (HIV) (11). In contrast, HIV replication has been shown to be inhibited in vitro by TGF-β (31) as well as by currently undefined noncytolytic factors produced by HIV-specific class I-restricted CTL upon antigen recognition (39). Furthermore, IL-1 and IL-6 are markedly elevated in the sera of HIV-infected patients (5), and IL-6 is also induced during acute and persistent lymphocytic choriomeningitis virus infection in mice (29). Moreover, cytokine-producing immune effector cells have been reported to control viral replication and contribute to viral clearance during lymphocytic choriomeningitis virus (24) and herpes simplex virus infection by noncytolytic mechanisms (25).

TABLE 1. Recombinant cytokines that do not reduce hepatic HBV mRNA[a]

Cytokine	Dosage	No. of daily injections
Human		
IL-1α	200,000 U	1
	500,000 U	1
	1,000,000 U	1
IL-6	500,000 U	1
	500,000 U	6
TGF-β	5 μg	1
Murine		
TNF-β	50,000 U	1
	100,000 U	1
	500,000 U	1
	1,000,000 U	1
GM-CSF	250 U	1
	1,200 U	1
	2,500 U	1
	4,000 U	1
IL-3	45,000 U	1
IFN-γ	100,000 U	1
	500,000 U	1
	100,000 U	6
	500,000 U	6

[a] Groups of three mice were injected either once or on 6 consecutive days with IL-1, IL-6, TGF-β, TNF-β, GM-CSF, IL-3, or IFN-γ at multiple dosage levels as indicated. None of these animals displayed any reduction of hepatic HBV mRNA 16 h following the last dose of each cytokine.

This study was undertaken to examine the capacity of selected inflammatory cytokines to modulate HBV gene expression, in the context of our interest in the role of the cellular immune response as a determinant of viral clearance and liver cell injury during HBV infection. Because of the complexity of the cytokine network in vivo, we used a transgenic mouse model, instead of a tissue culture system, in order to be able to identify the indirect as well as the direct effects of the inflammatory cytokines under investigation. This proved to be worthwhile because it permitted us to demonstrate that IL-2, a cytokine for which the hepatocyte lacks receptors (35), can down-regulate hepatocellular HBV gene expression by an indirect, noncytopathic process that is mediated by TNF-α. Since IL-2 is principally produced by activated class II-restricted, CD4-positive T cells, and since TNF-α is produced by class I-restricted, CD8-positive T cells, and especially by activated macrophages, these results suggest that the intrahepatic cellular immune response to HBV may play a hitherto unsuspected role in the biology of HBV infection by modulating HBV gene expression without destroying the infected cells.

These observations corroborate previous studies in which we demonstrated that recombinant TNF-α alone negatively regulates HBV gene expression in the same transgenic mouse lineage used in this study and in other lineages as well (15). Since a number of positively regulated acute-phase response genes, e.g., the metallothionein and serum amyloid A genes, were only slightly induced after IL-2 injection, while no significant change was observed in the expression of negatively regulated genes, such as the albumin gene (data not shown), it would appear that the negative effect of IL-2 on HBV mRNA content is more easily induced in the hepatocyte than are many of the other changes in hepatocellular gene expression during the acute-phase response. Since the expression of housekeeping genes (e.g., the GAPDH and β-actin genes) was unchanged following IL-2 administration (data not shown), it appears that the specific decrease in the content of HBV mRNA is not due to global suppression of the cellular transcriptional machinery. In support of this conclusion, we have recently determined that the regulatory effect of IL-2 and TNF-α on HBV gene expression occurs at the posttranscriptional level in these animals, suggesting that IL-2- and TNF-α-activated hepatocel-

lular genes may destabilize HBV mRNA in a relatively selective manner (17).

We assume that the TNF-α-dependent regulatory effect of IL-2 described in this report is mediated by intrahepatic macrophages that release TNF-α in response to activation by IL-2. If this assumption is correct, the IL-2–TNF-α pathway may represent an efficient mechanism to deliver a noncytopathic viral regulatory signal to the hepatocyte without activation of the multitude of TNF-α-responsive cells that are widely distributed throughout the body. This may also explain why we observed much lower induction of metallothionein mRNA content in the liver in response to IL-2 than TNF-α (Fig. 3). Since almost all cells in the body bear TNF-α receptors while the distribution of IL-2 receptors is much more limited, it is conceivable that the paradoxical differential activation of metallothionein is due to cytokines produced by TNF-α-responsive cells, within the liver or elsewhere in the body, that are not activated by IL-2.

Similar reasoning might explain why we did not observe a regulatory effect following the administration of IFN-γ, a well-known and powerful macrophage activator. This is particularly germane in view of our recent finding that intrahepatic HBV mRNA content is profoundly reduced in these animals following the injection of class I-restricted, HBsAg-specific CTL that secrete IFN-γ upon recognition of antigen and that this regulatory effect is quantitatively blocked by the prior administration of neutralizing antibodies specific for IFN-γ (16a). Collectively, these observations suggest that other IFN-γ-inducible gene products, activated within the liver or elsewhere, might counteract the negative regulatory effects of IFN-γ within the liver. Indeed, by delivering virus-specific effector cells to the site of viral replication, the immune response could release physiologically active concentrations of inflammatory cytokines locally, where they can exert their antiviral and pathogenetic effects without activating potentially confounding, even counterregulatory, effects elsewhere in the body.

Hepatic HBV gene expression was also down-regulated by IFN-α and IFN-β administration, but only when they were injected repetitively and at high dose levels. Interestingly, the effect of IFN-β was not counteracted by repetitive injection of a neutralizing dose of anti-TNF-α MAb (34), suggesting that IFN-α and IFN-β inhibit HBV gene expression by a TNF-α-independent pathway. While it is formally possible that the IL-2–TNF-α effect could be mediated by IFN-α and IFN-β, we believe that the profound differences in the kinetics and dose requirements for these two groups of cytokines make this an unlikely scenario. Although we do not know whether IFN-α and IFN-β reduce the steady-state content of HBV mRNA at the transcriptional or posttranscriptional level, the IFNs are well known to destabilize a number of viral RNAs by activating the oligoadenylate synthetase-RNase L system in responsive cells, which targets preferentially UU and UA motifs within susceptible transcripts (42). It will be interesting to determine whether this pathway is responsible for the down-regulatory effects of IFN-α and IFN-β in this model, especially in view of the widespread use of these agents for the treatment of chronic HBV infection in humans (18). Additionally, it would not be surprising to find that these two IFNs are produced within the infected liver, in view of the heterogeneous inflammatory infiltrate that characterizes viral hepatitis.

The specificity of IL-2, TNF-α, IFN-α, and IFN-β in this system is underscored by the fact that many other cytokines, including IL-1, IL-3, IL-6, TGF-β, TNF-β, and GM-CSF, did not affect HBV gene expression in these animals. All of these other cytokines are known to influence the expression of hepatocyte-, macrophage-, and/or endothelial cell-specific genes and to stimulate the release of other cytokines from correspondingly responsive target cells. Their failure to modulate HBV gene expression in this model may therefore reflect either their inability to activate the HBV-specific regulatory pathways that are activated by IL-2, TNF-α, IFN-α, and IFN-β in these animals or their ability to simultaneously activate potential counterregulatory pathways that have not yet been defined. While speculative at the moment, these alternatives represent interesting opportunities for future investigation.

Finally, it is important to emphasize that the regulatory effects of IL-2, TNF-α and the IFNs in this system were observed in the absence of any histopathological or biochemical evidence of liver cell injury. We have previously reported that IFN-γ is cytopathic for ground-glass hepatocytes in transgenic mice that overproduce the HBV large envelope polypeptide and accumulate filamentous HBsAg particles in the endoplasmic reticulum, apparently compromising the detoxification capacity of this organelle in the process (15). While we did not examine the effects of IFN-α or IFN-β in those earlier studies (15), it is important to emphasize that HBsAg is secreted, not retained, by the hepatocyte in the lineage of mice used in the current experiments so that the aforementioned hepatocytotoxic effect cannot occur in these animals. Additionally, it has been shown that recombinant TNF-α can be cytopathic for normal hepatocytes in vivo in certain strains of mice (14, 15) and that this cytokine is responsible for a significant component of the hepatocytotoxic effects of bacterial lipopolysaccharide in certain strains of mice previously treated with D-galactosamine or infected with *Corynebacterium parvus* or *Propionibacterium acnes* (10, 16, 38, 40). The lack of any evidence of liver disease following TNF-α administration in the mice used in this study, therefore, is probably due to an intrinsic resistance to the cytotoxic effects of TNF-α in these animals, presumably as a result of their genetic background (C57BL/6).

We conclude, therefore, that the regulatory effects of IL-2, TNF-α, IFN-α, and IFN-β occur independently of any role that they might play in the pathogenesis of liver cell injury during viral hepatitis. It is conceivable that by virtue of their regulatory effects, the cytokines produced by inflammatory cells in the HBV-infected liver might contribute either to viral clearance or, paradoxically, to evasion of the host immune response and progression to viral persistence. Additional studies will be required to address these important questions.

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