Inhibition of Hepatitis B Virus Replication during Schistosoma mansoni Infection in Transgenic Mice

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Abstract

Although coinfection of hepatitis B virus (HBV) and Schistosoma mansoni is a frequent event in humans, little is known about the interactions between these two pathogens. S. mansoni infection induces T helper cell type 2 (Th2)-type cytokines in the liver of humans and mice. The intrahepatic induction of nitric oxide (NO) and Th1-type cytokines, such as interferon (IFN)-γ and IFN-α/β, inhibits HBV replication noncytopathically in the liver of transgenic mice. To examine whether S. mansoni infection and the accompanying induction of Th2-type cytokines could interfere with HBV replication in the liver, HBV transgenic mice were infected with S. mansoni. By 5 wk after infection, HBV replication disappeared concomitant with the intrahepatic induction of NO and Th1-type cytokines, and in the absence of Th2-type cytokines. By 6–8 wk after infection, HBV replication remained undetectable and this was associated with further induction of NO and Th1-type cytokines together with the appearance of Th2-type cytokines. The S. mansoni-dependent antiviral effect was partially blocked by genetically deleting IFN-γ, although it was unaffected by deletion of IFN-α/β. These results indicate that IFN-γ (probably via NO) mediates most of this antiviral activity and that Th2-type cytokines do not counteract the antiviral effect of IFN-γ. Similar events may suppress HBV replication during human S. mansoni infection.

Key words: transgenic/knockout • infectious immunity virus • helminth parasites • Th1/Th2 cytokines • liver

Introduction

Hepatitis B virus (HBV) is a noncytopathic, double-stranded DNA virus that causes acute and chronic hepatitis and hepatocellular carcinoma in humans (1). It is widely believed that the cytotoxic T cell (CTL) response to the virus plays a critical role in viral clearance and liver disease. Indeed, the CTL response is strong, polyclonal, and multispecific during acute HBV infection, but is usually undetectable in patients with chronic hepatitis (1). Using a transgenic mouse model, we have shown recently that the antiviral potential of the CTLs is primarily mediated by noncytolytic mechanisms that involve the intrahepatic production of Th1-type cytokines such as IFN-γ (2, 3). We also showed that nitric oxide (NO) mediates most of the antiviral activity of IFN-γ produced by the CTLs and that IFN-α/β produced in the liver during unrelated hepatotropic virus infections inhibits HBV replication via NO-independent pathways (3–6). Noncytopathic antiviral mechanisms like these can contribute to viral clearance during acute viral hepatitis in chimpanzees, thus confirming the transgenic mouse studies in a natural infection model (7). All these events occur in the liver in the absence of Th2-type cytokines such as IL-4, IL-5, and IL-10. Whether the outcome of HBV infection in humans (viral clearance versus viral persistence) is influenced by qualitative differences in the intrahepatic cytokine profile (Th1-type versus Th2-type) is not known.

Schistosoma mansoni is a helminth parasite (class Trematoda) that infects both humans and mice. Schistosomiasis affects more than 200 million people worldwide, particularly in Africa, Asia, and South America, areas in which chronic HBV infection is endemic. Schistosomiasis is largely dependent on the abundance of the snail intermediate host. Infection begins when cercariae shed into the water by infected snails penetrate the skin of individuals exposed to contaminated water. After penetration, the larvae enter the microcirculation and reach the hepatic portal system where they remain. After several weeks of infection,
the female worm deposits a large number of eggs, leading to the formation of granulomas in the liver (8). The pathogenesis of egg granuloma formation involves a Th2-type cytokine response that begins few days after egg laying with production of increasing levels of IL-4, IL-5, and IL-10 by Th2 cells, eosinophils, and basophils (8, 9). In experimentally infected mice, the Th2-type cytokine response peaks on week 8 after infection, and this process is thought to downregulate the secretion of Th1-type cytokines (including TNF-α and IFN-γ) that peaks few weeks earlier (weeks 4–5) (8, 9). This shift in cytokine profile may initiate the chronic stage of infection and contribute to the amelioration of liver pathology by inhibiting the continuous production of potentially harmful inflammatory mediators (8, 9).

Although coinfection of HBV and S. mansoni is a frequent event in several geographic areas of the world, very little is known about the possible interactions between these two pathogens. In this study we took advantage of transgenic mice that replicate HBV at high levels in the liver to directly examine whether S. mansoni infection and the accompanying induction of Th2-type cytokines could modulate HBV replication. Thus, we monitored HBV replication in HBV transgenic mice that were infected with S. mansoni and we compared these results with those obtained in S. mansoni–infected HBV transgenic mice genetically deficient for IFN-γ or IFN-α/β receptor.

**Materials and Methods**

HBV Transgenic Mice. HBV transgenic mice from lineage 1.3.32 (inbred C57BL/6, official designation Tg[HBV 1.3 genome]Chi32) and lineage 1.3.46 (inbred B10D2, official designation Tg[HBV 1.3 genome]Chi46) were used in this study. Lineages 1.3.32 and 1.3.46 replicate high levels HBV in their livers without any evidence of cytopathology, as previously described (10). Lineage 1.3.32 were backcrossed against knockout mice that lack IFN-γ (11) and the IFN-α/β receptor (IFN-α/βR−/−) (12), as previously described (3). The knockout mice were provided by Drs Timothy Stewart (IFN-γ−/−) and Michel Aguet (IFN-α/βR−/−) at Genentech, South San Francisco, CA. The genetic background of the original parental lineages of IFN-γ−/− and IFN-α/βR−/− were 129/Sv/Ev × C57BL/6. The IFN-γ−/− mice were backcrossed four to five generations against BALBc and the IFN-α/βR−/− mice were backcrossed more than five generations against C57BL/6 before they were mated with lineage 1.3.46 (inbred B10D2). F2 progeny were interbred to yield hepatitis B e antigen-positive (HBeAg) F3 progeny that were either homozygous or heterozygous for the null mutation. In all experiments, the mice were matched for age (8 wk), sex (male), and HBeAg levels in their serum before experimental manipulations. All animals were housed in pathogen-free rooms under strict barrier conditions.

Infection of Mice with S. mansoni. 8-wk-old HBV transgenic male mice were infected by percutaneous exposure of tail skin for 60 min in water containing 20–25 cercariae, as previously described (13). Mice were killed at different weeks after infection and their livers were harvested for histological and histochemical analyses, or they were snap frozen in liquid nitrogen and stored at −80°C for subsequent molecular analyses (see below).
HBV replication in the liver of transgenic mice, four groups (six mice per group) of age- (8 wk), sex- (male), and serum HBeAg–matched transgenic mice from lineage 1.3.32 (inbred C57BL/6) were exposed to water containing cercariae and killed on weeks 5, 6, 7, and 8 after exposure. All animals were successfully infected as indicated by the presence of adult worms, eggs, and granulomas in the liver (see below).

Since each mouse in each group of six mice showed identical results, two representative mice per group are shown in Fig. 1. On week 5 after infection, HBV DNA replicative forms completely disappeared from the liver of transgenic mice when compared with saline-injected controls (Fig. 1). At this time point, the messages for the T cell markers CD3, CD4, and CD8, the macrophage marker F4-80, and the NK cell marker NK1.1 were induced, indicating that granulomas were already present in the liver, as confirmed by histological analysis (data not shown). Despite the presence of granulomas, no hepatocellular lysis was observed at this time, as underscored by the absence of serum ALT elevation (Fig. 1, bottom). The intrahepatic cytokine profile was marked directed towards a type 1 response, as indicated by the induction of iNOS, TNF-α, IL-1-α, IFN-γ, IL-1-β, and TGF-β. Furthermore, the messages for Th2-type cytokines were either not (IL-4 and IL-5) or barely (IL-10) induced (Fig. 1).

By weeks 6, 7, and 8 after infection, hepatic granulomas (Fig. 2 B) increased in number and size (data not shown).

Results and Discussion

Inhibition of HBV Replication during S. mansoni Infection. To determine whether S. mansoni infection could modulate HBV replication in the liver of transgenic mice, four groups (six mice per group) of age- (8 wk), sex- (male), and serum HBeAg–matched transgenic mice from lineage 1.3.32 (inbred C57BL/6) were exposed to water containing cercariae and killed on weeks 5, 6, 7, and 8 after exposure. All animals were successfully infected as indicated by the presence of adult worms, eggs, and granulomas in the liver (see below).

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and HBV replication remained suppressed. Since viral replication occurs inside of HBcAg-positive nucleocapsid particles in the cytoplasm of centrilobular hepatocytes (Fig. 2A) in these transgenic mice (10), it is not surprising that HBcAg disappeared from the cytoplasm of these cells (Fig. 2B), along with the disappearance of HBV replicative forms (Fig. 1). The intrahepatic expression of iNOS, Th1-type cytokines, and T lymphocyte, macrophage, and NK cell markers progressively increased, reaching its peak by week 8 after infection. This was accompanied by the induction of Th2-type cytokines (IL-4, IL-5, and IL-10) that started by week 6 and also peaked by week 8 (Fig. 1). These results were somewhat surprising since others have reported that the peak of Th2-type cytokine response after S. mansoni infection in mice (week 8) coincides with the downregulation of Th1-type cytokines (for review see reference 8). Using a quantitative assay (R Nase Protection) to measure the intrahepatic content of cytokine messages, we confirmed in this study that Th2-type cytokines peaked on week 8, but this did not coincide with any waning of the type 1 immune response in the liver, which also peaked by week 8 (Fig. 1).

No induction of 2′5′-OAS (a marker of IFN-α/β induction) was detected throughout the infection, indicating that IFN-α/β is not produced in the liver of S. mansoni-infected animals. sALT activity slightly increased by week 6, peaked at week 7, and started to decline by week 8 (Fig. 1, bottom). Given the relatively low levels of sALT, the extent of hepatocellular lysis in these animals was moderate throughout the infection.

Groups of HBV transgenic mice (three mice per group) were also killed during the chronic stage of infection (weeks 10 and 12). The number of hepatic granulomas decreased, Th1-type and Th2-type cytokines were still induced in the liver (although to lower levels compared with week 8), and HBV replication remained downregulated (data not shown). Finally, similar results were obtained when groups (four mice per group) of age- (8 wk), sex- (male), and serum HBeAg–matched transgenic mice from lineage 1.3.46 that were either heterozygous (1/2) or homozygous (1/1) for the IFN-γ and the IFN-α/β receptor null mutations were exposed to water containing cercariae and killed on week 8 after exposure. Total hepatic DNA was analyzed for HBV DNA by Southern blot (SB) analysis. All DNA samples were RNAse treated before gel electrophoresis. Bands corresponding to the integrated transgene (Transg.), relaxed-circular (RC), and single-stranded (SS) linear HBV DNA replicative forms are indicated. The integrated transgene can be visualized by hybridization with a 32P-labeled HBV-specific DNA probe. Total hepatic RNA was analyzed for HBV DNA by Northern blot (NB) analysis and for the message of iNOS, various cytokine transcripts, CD3, CD4, CD8, F480, and NK1.1 by RNase Protection assay (RPA), as indicated. The mRNA encoding the ribosomal protein L32 was used to normalize the amount of RNA loaded in each lane of the RPA assay. Results were compared with those observed in livers pooled from six age-, sex-, and serum HBsAg-matched transgenic mice (six mice per group) that were either homozygous (1/1) or heterozygous (1/2) for these null mutations were exposed to water containing cercariae and killed on week 8 after exposure.

As shown in Fig. 3 for three representative mice per group, HBV DNA replicative forms disappeared from the liver of transgenic mice (10), it is not surprising that HBcAg disappeared from the cytoplasm of these cells (Fig. 2B), along with the disappearance of HBV replicative forms (Fig. 1). The intrahepatic expression of iNOS, Th1-type cytokines, and T lymphocyte, macrophage, and NK cell markers progressively increased, reaching its peak by week 8 after infection. This was accompanied by the induction of Th2-type cytokines (IL-4, IL-5, and IL-10) that started by week 6 and also peaked by week 8 (Fig. 1). These results were somewhat surprising since others have reported that the peak of Th2-type cytokine response after S. mansoni infection in mice (week 8) coincides with the downregulation of Th1-type cytokines (for review see reference 8). Using a quantitative assay (R Nase Protection) to measure the intrahepatic content of cytokine messages, we confirmed in this study that Th2-type cytokines peaked on week 8, but this did not coincide with any waning of the type 1 immune response in the liver, which also peaked by week 8 (Fig. 1).

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Figure 3. Suppression of HBV replication by S. mansoni infection is mostly mediated by IFN-γ. Age-, sex-, and serum HBsAg-matched transgenic mice from lineage 1.3.46 that were either heterozygous (1/2) or homozygous (1/1) for the IFN-γ and the IFN-α/β receptor null mutations were exposed to water containing cercariae and killed on week 8 after exposure.

Total hepatic DNA was analyzed for HBV DNA by Southern blot (SB) analysis. All DNA samples were RNAse treated before gel electrophoresis. Bands corresponding to the integrated transgene (Transg.), relaxed-circular (RC), and single-stranded (SS) linear HBV DNA replicative forms are indicated. The integrated transgene can be used to normalize the amount of DNA bound to the membrane. The filter was hybridized with a 32P-labeled HBV-specific DNA probe. Total hepatic RNA was analyzed for the message of 2′5′-OAS and GAPDH by Northern blot (NB) analysis and for the message of iNOS, various cytokine transcripts, CD3, CD4, CD8, F480, and NK1.1 by RNase Protection assay (RPA), as indicated. The RNA encoding the ribosomal protein L32 was used to normalize the amount of RNA loaded in each lane of the RPA assay. Results were compared with those observed in livers pooled from six age-, sex-, and serum HBsAg-matched, saline-injected transgenic controls (time 0). The mean sALT activity, measured at the time of autopsy, is indicated at the bottom for each group and is expressed in units per liter.
liver of all of the heterozygous mice, coinciding with induction of iNOS, Th1- and Th2-type cytokines, and T lymphocyte, macrophage, and NK cell markers. Similarly, HBV replication was abolished in the liver of IFN-α/βR−/− mice, indicating that IFN-α/β does not contribute to the antiviral effect of S. mansoni infection. This is compatible with the fact that 2′5′-OAS was not induced in the S. mansoni−infected livers (Figs. 1 and 3).

In contrast, HBV replication was only partially reduced in the IFN-γ knockout animals (Fig. 3), indicating that IFN-γ mediates most of the antiviral activity of S. mansoni infection. It is noteworthy that the hepatic levels of HBV replicative forms were quite variable among different individuals (the variability in HBV DNA content extended to three additional mice that are not shown in Fig. 3). The reason for this variability remains to be determined. Nevertheless, HBV DNA remained detectable in all IFN-γ−/− mice and, in keeping with this, HBcAg also remained detectable in the cytoplasm of hepatocytes, even in those that were directly adjacent to hepatocellular granulomas (Fig. 2 C).

Interestingly, the intrahepatic content of iNOS mRNA was markedly reduced in the liver of IFN-γ−/− mice (approximately fivefold, as measured by phosphorimaging analysis; data not shown), as compared with the liver of all of the other S. mansoni−infected mice (Fig. 3). The fact that IFN-γ−/− mice showed reduced intrahepatic levels of iNOS at this time point suggests that NO may have contributed to the antiviral effect of IFN-γ in this system. This would be consistent with a previous report from our laboratory where it was shown that virus−specific CTLs can abolish HBV replication in the hepatocytes of transgenic mice by IFN-γ−/− mice (23). The results of this study indicate that IFN-γ inhibits HBV replication in the presence of IL-4, IL-5, and IL-10. Therefore, it is possible that persistence of HBV may primarily result from a quantitative deficiency of Th1-type responses rather than qualitative differences in the intrahepatic cytokine profile. Accordingly, it has been shown recently that the number of IFN-γ−producing antigen−specific T cells isolated from the peripheral blood of people chronically infected with HBV is much lower than that observed in the peripheral blood of people acutely infected with HBV (24).

We thank Alan Sher for helpful discussion; Fred Lewis for providing Schistosome life cycle stages supplied through National Institutes of Health–N ational Institute of Allergy and Infectious Diseases contract N01-A1-55270; Timothy Stewart and Michel Aguet for providing IFN-γ−/− and IFN-α/βR−/− mice, respectively; Ian Campbell for providing the iNOS probe, and Monte Hobbs for providing the cytokine gene and T cell marker probe sets used in the R NASE Protection assays; and Margie Chadwell for excellent technical assistance.

This work was supported by grants AI-40696 (to L.G. Guidotti) and CA-40489 (to F.V. Chisari) from the National Institutes of Health. This is manuscript number 13090-M EM from the Scripps Research Institute.

Submitted: 22 February 2000
Revised: 22 March 2000
Accepted: 24 April 2000

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