Host–Virus Interactions during Malaria Infection in Hepatitis B Virus Transgenic Mice

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Abstract

We have previously shown that hepatitis B virus (HBV) replication is abolished in the liver of HBV transgenic mice by inflammatory cytokines induced by HBV-specific cytotoxic T cells and during unrelated viral infections of the liver. We now report that intrahepatic HBV replication is also inhibited in mice infected by the malaria species *Plasmodium yoelii* 17X NL. *P. yoelii* infection triggers an intrahepatic inflammatory response characterized by the influx of natural killer cells, macrophages, and T cells. During this process, interferon (IFN)-γ and IFN-α/β suppress HBV gene expression and replication in the liver. Collectively, the data suggest that malaria infection might influence the course and pathogenesis of HBV infection in coinfected humans.

Key words: hepatitis B virus transgenic mice • *Plasmodium yoelii* • inflammatory cells • inflammatory cytokines • liver

Introduction

The hepatitis B virus (HBV)[1] is a noncytopathic, enveloped virus that causes acute and chronic hepatitis and hepatocellular carcinoma (1). Viral clearance and liver disease during acute HBV infection are associated with a strong, polyclonal, and multispecific CTL response, which is weak or not detectable in patients with chronic hepatitis (1). We have reported previously that IFN-γ produced by hepatitis B surface antigen–specific CTLs is sufficient to inhibit HBV replication in the liver of HBV transgenic mice (2–4), and that this process is mediated by nitric oxide (NO) (5). We have also demonstrated that HBV replication can be abolished in these mice in response to inflammatory cytokines, especially IFN-α/β and IFN-γ that are produced in the liver during lymphocytic choriomeningitis virus (2, 6), murine cytomegalovirus, and adenovirus (2, 7) infections. Moreover, we have recently shown that similar cytokine-dependent, noncytopathic antiviral mechanisms can contribute to viral clearance during acute viral hepatitis in chimpanzees by purging HBV replicative intermediates from the cytoplasm and covalently closed circular viral DNA from the nucleus of infected cells (8). The current study was performed to see if a similar antiviral process is induced during *Plasmodium yoelii* infection in HBV transgenic mice, since malaria and HBV coinfection is common in many parts of the world.

Malaria is initiated by 1–10 sporozoites injected into the host by a female anopheles mosquito. After a short time in the circulation, each sporozoite infects a hepatocyte within which they proliferate and develop into the liver stages of the plasmodial life cycle (9, 10). Eventually, the infected hepatocytes rupture, releasing hundreds of merozoites that infect RBCs and initiate the blood stage of the infection that rapidly spreads and can destroy up to 50% of the RBCs before the infection is controlled by the immune response (11). Because merozoites do not infect hepatocytes, the infection is usually self-limited if the blood stage is controlled.

The hepatic and blood stages of malaria are controlled by Abs, macrophages, and cytokines (12–17) including IFN-γ via the induction of NO, TNF-α, IL-1, and IL-6 (18–22). Th1 and Th2 responses are induced during the blood stage infection, and the balance between these two subsets plays a vital role in the outcome of the infection, with a Th1 response appearing to be essential for elimination of the parasites (23–26). The hepatic stage of the infection is also controlled by CTLs (14–16).

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[1]*Abbreviations used in this paper:* GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; iNOS, inducible nitric oxide synthase; 2′5′-OAS, 2′5′-oligoadenylate synthetase; sALT, serum alanine aminotransferase.

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While coinfection by HBV and malaria is a major public health problem in large areas of the world, the mutual interactions between the two pathogens are poorly understood. Because inflammatory cytokines are induced during malaria and contribute to its control, it is possible that the same cytokines could interfere with HBV replication in the liver in coinfected individuals. Indeed, high levels of TNF-α, IFN-γ, and IL-6 are detectable in the blood of malaria patients and in the spleen and liver in rodent models of malaria (11, 27–32). Furthermore, parasitized RBCs are cleared by resident hepatic and splenic macrophages (33, 34) that can not only secrete antiviral cytokines but also function as antigen presenting cells, and produce chemokines that lead to recruitment of T cells that can secrete their own salvo of antiviral cytokines in the infected liver.

Since inflammatory cytokines are known to suppress HBV replication in HBV transgenic mice (3), we predicted that HBV replication in these animals might be inhibited during *P. yoelii* infection. This study was performed to test that prediction.

Materials and Methods

Mice. HBV transgenic mouse lineages 1.3.32 and 1.3.46 used in this study have been described previously (35). Both lineages of mice replicate HBV at high levels in the liver and kidney without any evidence of cytopathology. Lineage 1.3.32 was derived from an outbred founder. It was expanded by repetitive backcrossing for more than 10 generations against C57BL/6, and then bred 1 generation against BALB/c mice to produce F_1 hybrids that were used in this study. Lineage 1.3.46 was derived from an inbred B10D2 founder and has been continually expanded on that background. In the current study, lineage 1.3.46 was backcrossed against a panel of mice whose IFN-γ (IFN-$\gamma^{-/-}$) (36), IFN-α/β receptor (IFN-α/β$R^{-/-}$) (37), TNF-αp55 receptor (TNF-α$R^{-/-}$) (38), or inducible nitric oxide synthase (iNOS) (i$NOS^{-/-}$) (39) alleles had been knocked out, exactly as described (2, 5). The IFN-$\gamma^{-/-}$ and IFN-α/β$R^{-/-}$ mice were provided, respectively, by Timothy Stewart and Michel Aguet (Genentech, Inc., South San Francisco, CA). The TNF-α$R^{-/-}$ mice were provided by Tak Mak (University of Toronto, Ontario, Canada). The i$NOS^{-/-}$ mice were provided by John Mudgett (Merck Research Laboratories, Rahway, NJ) and John MacMicking and Carl Nathan (Cornell University Medical College, New York, NY). The genetic background of the original knockout animals was 129/Sv/Ev/ × C57BL/6. Before mating with the HBV transgenic mice, the IFN-$\gamma^{-/-}$ mice had been backcrossed four to five generations against BALB/c and the IFN-α/β$R^{-/-}$, and the TNF-α$R^{-/-}$ and i$NOS^{-/-}$ mice had been backcrossed more than five generations against C57BL/6. The serum from the 1.3.46 × knockout F_1 progeny was tested for hepatitis B e antigen (HBeAg)-positive using a commercially available kit (Abbott Laboratories). HBeAg-positive F_1 mice were backcrossed to the corresponding parental knockout lineage to produce HBeAg-positive F_2 progeny that were either homozygous or heterozygous for the null allele that was distinguishable by allele-specific PCR reactions, as described previously (36–39). Thus, the HBV × knockout animals were genetically heterogeneous with variable contributions from BALB/c, B10D2, and C57BL/6 parental strains. In all experiments, the mice were matched for age (8 wk), sex (male), and serum HBeAg levels before experimental manipulation. All animals were housed in pathogen-free rooms under strict barrier conditions.

P. yoelii Infection. The *P. yoelii yoelii* (17X NL nonlethal strain) was maintained by alternating passage of the parasites in *Anopheles stephensi* mosquitoes and in B10.D2 mice, as described previously (40, 41). Sporozoites were collected 14 d after an infectious blood meal by hand dissection of *P. yoelii* 17 XNL–infected mosquito salivary glands in M199 medium containing 1% normal mouse serum (Rockland, Inc.) as described previously (42). Erythrocytic stage parasites were obtained from heparinized blood of a previously sporozoite-infected mouse, and maintained by cyclic passages of the parasites in congenic mice, as described previously (43). The parasitized RBCs were collected in equal volume of HBSS (GIBCO BRL) plus 20% glycerol (Sigma-Aldrich) plus 100 U/ml heparin (Sigma-Aldrich), and kept in liquid nitrogen. Mice were injected either intravenously with 10^6 sporozoites or intraperitoneally with 10^7 parasitized RBCs. Control mice were injected either with an extract of uninfected salivary glands or with uninfected RBCs, respectively. Animals were killed at multiple time points after infection, and their livers were harvested for histological, immunohistochemical, and histochemical analyses (see below), or they were snap frozen in liquid nitrogen and stored at –80°C for subsequent DNA and RNA analyses. Parasitemia was determined by microscopic examination of Giemsa-stained thin blood smears, obtained from days 1–30 after inoculation.

Tissue DNA and RNA Analyses. Frozen liver (left lobe) was mechanically pulverized under liquid nitrogen, and total genomic DNA and RNA were isolated for Southern and Northern blot analyses exactly as described previously (35). Nylon membranes were analyzed for HBV DNA, HBV RNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and 2′5′-oligoadenylate synthetase (2′5′-OAS) as described elsewhere (4). Quantification of iNOS, cytokine, T lymphocyte, and macrophage and neutrophil marker mRNAs was performed by RNase Protection assay exactly as described previously (4, 8, 44). A fragment (nucleotides 124–368) from the mNK1.1 cDNA (Genbank accession number S43141) was subcloned into a PGEM-T Easy vector (Promega) using PCR-assisted cloning (44). The vector was linearized and used for T7 polymerase–directed synthesis of a ^{32}P-labeled antisense RNA probe. The hybridization reactions, RNase treatments, isolations of protected RNA duplexes, and resolution of protected probes by denaturing PAGE gels were carried out as described (44). Dried gels were placed on film (XAR; Eastman Kodak Co.) with intensifying screens for various periods of time at −70°C.

Biochemical and Histological Analysis. The extent of hepatocellular injury during malaria infection was monitored histologically and biochemically by measuring serum alanine aminotransferase (sALT) activity at multiple time points after infection. sALT activity was measured in a Paramax chemical analyzer (Baxter Diagnostics, Inc.) exactly as described previously (4). For histological analysis, liver tissue was fixed in 10% zinc-buffered formalin (Anatech), embedded in paraffin, sectioned (3 μm), and stained with hematoxylin and eosin.

Results and Discussion

P. yoelii Infection: Parasitemia and Liver Disease in HBV Transgenic Mice. 21 age-, sex-, and serum HBeAg-matched transgenic mice from lineage 1.3.32 were infected either intravenously with 10^6 *P. yoelii* sporozoites (liver stage), or intraperitoneally with 10^7 parasitized RBCs

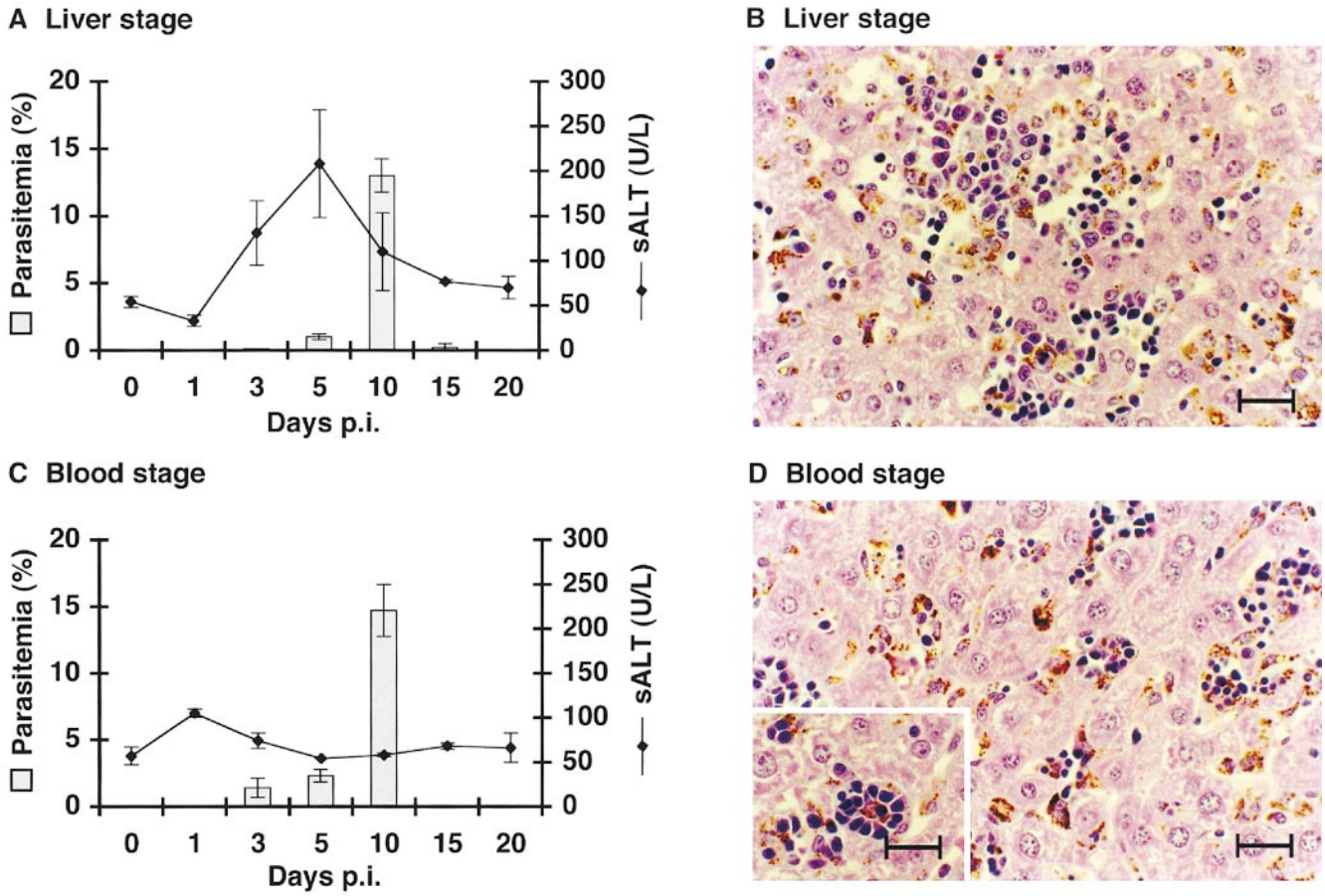

Figure 1. Parasitemia and liver disease after *P. yoelii* infection of HBV transgenic mice. Age-, sex-, and serum HBeAg-matched HBV transgenic mice (lineage 1.3.32) were injected either intravenously with 10^6 sporozoites (liver stage), or intraperitoneally with 10^7 parasitized RBCs (blood stage) of *P. yoelii yoelii* strain 17X NL, and three mice per group were killed on days 1, 3, 5, 10, 15, and 20 after infection, as indicated. Control mice (time 0) were injected with either uninfected salivary glands or uninfected RBCs. The mean percentage of blood parasitemia and the mean sALT activity (expressed in U/liter), measured at the time of autopsy, are indicated for each group of mice (A and C). Hematoxylin and eosin–stained liver sections (original magnification: ×400; bar, 25 μm) from animals killed 10 d after either liver stage infection (B) or blood stage infection (D) was performed. Inset, original magnification: ×600; bar, 35 μm. These experiments have been reproduced twice for the liver stage and three times for the blood stage with identical results. p.i., post infection.

(blood stage), and groups of three mice were killed on days 1, 3, 5, 10, 15, and 20 after infection. Control mice were injected with either uninfected salivary glands or uninfected RBCs. The infected animals developed a time-dependent necroinflammatory liver disease that was detectable histologically starting 1 d after infection and lasted for 10–15 d until the parasites were no longer detectable in the blood on day 20 (Fig. 1 A). In keeping with the fact that 10^6 sporozoites cannot infect more than 10^6 hepatocytes (1% of the hepatocyte population) and that the blood stage parasites do not infect hepatocytes, very few hepatocytes were destroyed during the infection. This was reflected in the very slight elevation in serum sALT activity that was detectable between days 3 and 10 after liver stage inoculation, and the even smaller sALT elevation between days 1 and 3 after blood stage inoculation (Fig. 1 A). At the peak of parasitemia in both types of infection (day 10), many of the hepatic macrophages (Kupffer cells) were filled with malaria pigment derived from ruptured infected erythrocytes, and at this time a large number of inflammatory foci consisting of Kupffer cells and lymphomononuclear cells were widely distributed throughout the liver (Fig. 1, B and C). Numerous pigment-laden macrophages surrounded by lymphomononuclear cells were present (Fig. 1 C, inset). The histological features of the disease are strikingly similar in both the sporozoite and merozoite infections, suggesting that the inflammatory process in the sporozoite-infected animals was probably due primarily to the blood stage of that infection, although small numbers ($\sim 10^6$ or $\sim$1% of total) of hepatocytes were destroyed by the sporozoites in the liver stage infection, as suggested by the slightly higher sALT activity in that infection (Fig. 1 A). Finally, the histopathological features of the liver disease during either the liver or the blood stage of infection were not influenced by the genetic background of the animals, since in independent experiments we showed very similar results after malaria infection of C57BL/6, B10.D2, and BALB/c mice (not shown).

P. yoelii Infection: Effect on HBV Gene Expression and Replication. Northern and Southern blot analyses were performed to compare the levels of HBV RNA and replicative

HBV DNA forms in *P. yoelii*–infected mice and in age- and sex-matched transgenic controls. After extraction, total hepatic RNA and DNA from three mice per group were pooled for analysis. As shown in Fig. 2 (A and B), hepatic HBV RNA and replicative DNA forms were slightly reduced on day 1 after liver stage infection while they were unchanged at this time after blood stage infection. Correspondingly, 2′5′-OAS mRNA, a type 1 IFN-inducible gene, TNF-α mRNA, iNOS mRNA, as well as the macrophage marker F4/80 were slightly induced in the liver stage but not the blood stage infection, and IFN-γ, CD3, CD4, CD8, and NK1.1 mRNAs were not increased (Fig. 2 C). These results indicate that macrophages are the likely source of the IFN-α/β, TNF-α, and iNOS detected on day 1, especially since they are known to produce these factors upon activation (45).

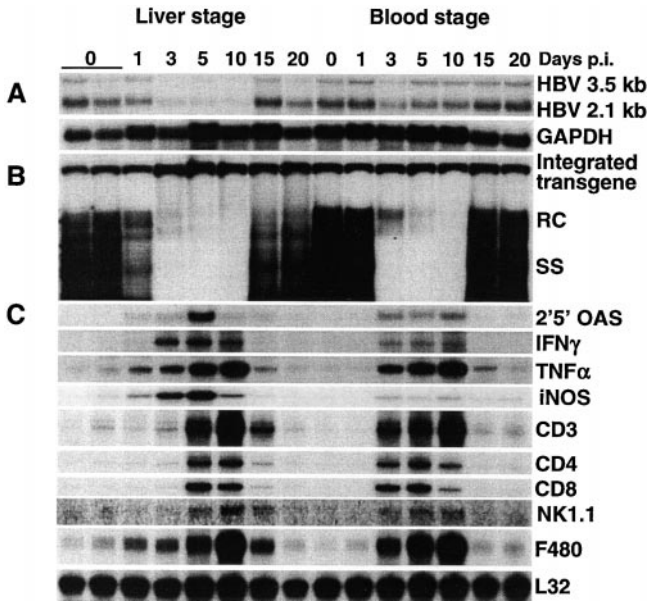

Figure 2. *P. yoelii* infection inhibits hepatic HBV replication and induces the expression of cytokine genes and inflammatory-cell markers in the liver. Age-, sex-, and serum HBeAg-matched HBV transgenic mice (lineage 1.3.32) were injected either intravenously with 10^6 sporozoites (liver stage) or intraperitoneally with 10^7 parasitized RBCs (blood stage) of *P. yoelii yoelii* strain 17X NL, and livers were harvested from each of three mice killed on days 1, 3, 5, 10, 15, and 20 after infection, as indicated. Control mice (time 0) were injected with either uninfected salivary glands or uninfected RBCs. Northern blot analysis (A) was performed with total liver RNA pooled from three mice in each group. The membrane was cohybridized with ^{32}P-labeled HBV– and GAPDH–specific DNA probes. The steady-state HBV and GAPDH mRNA content was compared with total hepatic RNA from control mice. Bands corresponding to the 3.5- and 2.1-kb HBV mRNAs are indicated. Southern blot analysis (B) was performed with 30 μg of total liver DNA isolated from the same pool of mice. All DNA samples were treated with RNase A before their concentrations were determined. Bands corresponding to the integrated transgene and the relaxed circular (RC) and single-stranded (SS) HBV DNA replicative forms are indicated. The integrated transgene can be used to normalize the amount of DNA bound to the membrane. The membrane was hybridized with a ^{32}P-labeled HBV–specific DNA probe. Total liver RNA from the same mice was analyzed by Northern blot analysis for the expression of 2′5′-OAS (C), a marker of IFN-α/β induction. The housekeeping enzyme GAPDH was used to normalize the amount of RNA loaded in each lane, and its expression was uniform in all mice (not shown). Total hepatic RNA from the same *Plasmodium*-infected transgenic mice was also analyzed by RNase Protection (C) for the expression of IFN-γ, TNF-α, and iNOS transcripts, and for the expression of CD3γ, CD4, CD8, NK1.1, and F4/80, as indicated. The mRNA encoding the ribosomal protein L32 was used to normalize the amount of RNA loaded in each lane. These experiments have been reproduced twice for the liver stage and three times for the blood stage with identical results.

By day 3, hepatic HBV RNA and DNA were strongly reduced in both liver stage and blood stage infected mice. In the liver stage infection, this was accompanied by a strong induction of IFN-γ and iNOS mRNA and a slight further increase in 2′5′-OAS and TNF-α mRNA even though T cell markers were unchanged and NK cell markers were only slightly increased. In contrast, on day 3 of the blood stage infection, IFN-γ and iNOS mRNAs were much less strongly induced than in the liver stage infection, even though the T cell and macrophage markers were more strongly induced. In addition, 2′5′-OAS and NK cell mRNAs were relatively more abundant in these mice than in the liver stage infection. The relatively strong induction of IFN-γ after liver stage infection suggests that the resident and/or recruited intrahepatic population of NK, NKT, and T cells may have become activated to produce the cytokines at this time. The induction of iNOS mRNA suggests that the macrophages were further activated at this time point, presumably due to the increased levels of IFN-γ in the liver.

On day 5 after both infections, HBV replication and HBV gene expression remained suppressed, especially in the liver stage infection where 2′5′-OAS mRNA was very strongly induced, and where NK1.1 mRNA was more prominent and T cell markers were strongly enhanced. Not surprisingly, this was associated with the continued expression of iNOS, IFN-γ, and TNF-α. In both infections, the T cell and macrophage response, as well as IFN-γ and TNF-α gene expression, continued through day 10 when the parasitemia was at its peak (Fig. 1 A) and HBV replication was most profoundly suppressed. By day 15, the parasites were almost completely cleared from the circulation, and cytokine, macrophage, T cell markers, and sALT activity returned toward baseline levels, as did the hepatic content of HBV DNA and HBV RNA. All of these events were completed by day 20.

In summary, while the histological features of the two infections are quite similar, the liver stage infection appeared to trigger an early T cell–independent cytokine response as well as a delayed cytokine response that coincided with the infiltration of T cells. In contrast, the T cell response occurred earlier in the blood stage than in liver stage infection, perhaps because parasitemia was detectable earlier in those animals, and a T cell–independent phase was not observed, perhaps indicating that it was induced primarily by infected hepatocytes. In both cases, however, the induced cytokine responses were associated with a decrease in HBV RNA and DNA in the liver.

P. yoelii–induced Suppression of HBV Replication Is Mediated by IFNs. Since IFN-γ, TNF-α, 2′5′-OAS, and iNOS gene expression coincided with HBV downregulation in

the foregoing experiment, and since we have previously shown that the corresponding cytokines and NO inhibit HBV replication under other experimental conditions (2–7), we monitored the ability of *P. yoelii* merozoites to inhibit HBV replication in HBV transgenic mice whose ability to produce IFN-γ or iNOS, or to respond to TNF-α or IFN-α/β had been knocked out. Groups of four age-, sex-, and HBeAg-matched transgenic mice that were either homozygous or heterozygous for each of these null alleles were injected intraperitoneally with 10^7 merozoite-infected RBCs to initiate a blood stage infection. 10 d after infection, their livers were harvested and total hepatic DNA and RNA were extracted and subjected to Southern blot analysis for HBV DNA, Northern blot analysis for 2′5′-OAS mRNA, and RNase Protection assays for cytokine, F4/80, and T cell mRNA content. Since each mouse in each group of four mice showed identical results, two representative mice per group are shown in Fig. 3. As expected, HBV replication was abolished in all of the heterozygous control mice (represented by the IFN-$\alpha/\beta^{+/-}$ heterozygous animals in Fig. 3 A), coinciding with induction of 2′5′-OAS, TNF-α, IFN-γ, and T cell mRNAs (Fig. 3 B). Importantly, HBV replication was not suppressed in the IFN-γ knockout mice, despite the induction of 2′5′-OAS; it was only slightly suppressed in the absence of the IFN-α/β receptor, despite the induction of IFN-γ. These results suggest that IFN-γ and IFN-α/β act independently to inhibit HBV replication after *P. yoelii* infection. Surprisingly, HBV replication was inhibited in the absence of both iNOS and the TNFp55 receptor, indicating that neither NO nor TNF-α receptor–mediated signaling contributes substantially to the antiviral effect of the merozoites as long as IFN-α/β and IFN-γ are expressed in the liver. The surprising apparent irrelevance of TNF-α might be explained by the fact that 2′5′-OAS (i.e., IFN-α/β) and IFN-γ are hyperinduced in these animals, suggesting that, at these levels, the IFNs might compensate for the absence of TNF-α and be sufficient to downregulate HBV independently. The fact that the IFN-γ–associated antiviral effect was not seen in the iNOS knockout mice (Fig. 3) was also surprising, since the IFN-γ–dependent inhibition of HBV replication by CTLs is mediated by NO (4). This is compatible with two interpretations. First, the antiviral effect in those animals could be mediated by IFN-α/β since the 2′5′-OAS mRNA was strongly induced, and we have shown that the antiviral effect of IFN-α/β is NO independent. Alternatively, the IFN-γ–dependent antiviral effect triggered by *P. yoelii* might not be mediated by NO. This would be consistent with reports that certain IFN-γ–dependent effects are mediated by NO-independent mechanisms (46, 47). This putative activity could be enhanced by the fact that IFN-γ and TNF-α appear to be hyperinduced in these mice.

In summary, we report that HBV replication and gene expression are inhibited during *P. yoelii* infection which ultimately clears HBV virions from the liver by triggering an inflammatory response and the induction of IFN-γ, IFN-α/β, TNF-α, and iNOS in the liver. While all of these cytokines probably function collectively to inhibit HBV replication, it appears that IFN-γ and IFN-α/β are essential, and that TNF-α and iNOS are dispensable for the antiviral effect to occur. Since merozoite infection is sufficient to inhibit HBV replication in these mice, and since hepatocytes are not infected by the blood stages of the parasite, the antiviral effect of malaria is likely to be triggered principally by phagocytosis of infected erythrocytes by Kupffer cells, leading to their activation and production of chemokines that recruit NK cells, NKT cells, and malaria-specific T cells, all of which probably produce inflammatory cytokines that eliminate HBV from the hepatocytes.

These results suggest that similar antiviral events may limit HBV infection during human malaria infection, especially

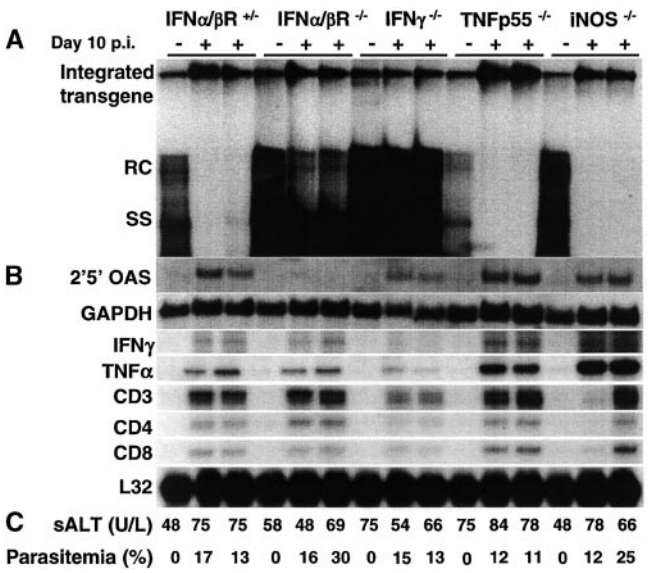

Figure 3. Early suppression of HBV replication by malarial infection is mediated by IFNs. Groups of four age-, sex-, and serum HBeAg-matched HBV transgenic mice (lineage 1.3.46) knocked out for IFN-γ, IFN-α/β receptor, TNF-α, or iNOS genes were injected intraperitoneally with 10^7 parasitized RBCs of *P. yoelii yoelii* strain 17X NL, and livers were harvested from each of four mice killed on day 10 after infection. Southern blot analysis (A) was performed with total liver DNA isolated from two representative animals in each group. The steady-state HBV content was compared with total hepatic DNA pooled from four control mice injected with uninfected RBCs (−). All DNA samples were treated with RNase A before their concentrations were determined. Bands corresponding to the integrated transgene and the relaxed circular (RC) and single-stranded (SS) HBV DNA replicative forms are indicated. The integrated transgene can be used to normalize the amount of DNA bound to the membrane. The membrane was hybridized with a ^{32}P-labeled HBV–specific DNA probe. Total liver RNA (20 μg) from the same pool of mice was analyzed by Northern blot analysis for the expression of 2′5′-OAS (B), a marker of IFN-α/β induction. The housekeeping enzyme GAPDH was used to normalize the amount of RNA loaded in each lane. Total hepatic RNA (5 μg) from the same *Plasmodium*-infected transgenic mice was also analyzed by RNase Protection (B) for the expression of IFN-γ, TNF-α, and iNOS transcripts, and for the expression of CD3γ, CD4, CD8, NK1.1, and F4/80, as indicated. The mRNA encoding the ribosomal protein L32 was used to normalize the amount of RNA loaded in each lane. The sALT activity, measured at the time of autopsy, is indicated for each mouse and is expressed in U/liter. The percentage of blood parasitemia, measured at the time of autopsy, is indicated for each mouse. Similar results were obtained in two separate experiments.

in endemic areas where both pathogens coexist at high frequency. Indeed, in a study investigating the interactions between malaria and hepatitis B in Papua New Guinea, it has been shown that patients with the highest rates of malaria infection had the lowest prevalence of hepatitis B infection (48). It is also noteworthy that viremia in chronic hepatitis B infection appears to fluctuate in concert with *Plasmodium falciparum* infection (49), suggesting that the amount and type of intrahepatic inflammatory cytokines induced by malaria infection may influence the course of HBV infection in man. Furthermore, mice infected with nonlethal strains of *P. yoelii* and *Plasmodium chabaudi* have been shown to produce a stronger IFN-γ response to infection than mice infected with the respective lethal strains (23), suggesting that the amount and quality of the inflammatory cytokines induced in the liver might control those infections.

In conclusion, these results suggest that the inflammatory cytokines induced and/or secreted by activated macrophages and the inflammatory cells they recruit into the liver during malaria infection may play a pivotal role in the outcome of coexisting HBV infection. These results not only provide insight into the possible impact of malaria on HBV replication in coinfected humans, they also suggest that pharmacological strategies designed to activate the intrahepatic macrophage may have therapeutic merit during chronic HBV infection.

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