Novel woodchuck hepatitis virus (WHV) transgene mouse models show sex-dependent WHV replicative activity and development of spontaneous immune responses to WHV proteins

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Abstract

The woodchuck model is an informative model for studies on hepadnaviral infection. In this study, woodchuck hepatitis virus (WHV) transgenic (Tg) mouse models based on C57BL/6 mice were established to study the pathogenesis associated with hepadnaviral infection. Two lineages of WHV Tg mice harboring the WHV wild-type (1217) and a mutated WHV genome lacking surface antigen (1281) were generated. WHV replication intermediates were detected by Southern blotting. DNA vaccines against WHV proteins were applied by intramuscular injection. WHV-specific immune responses were analyzed by flow cytometry and ELISA. The presence of WHV transgenes resulted in liver-specific but sex- and age-dependent WHV replication in Tg mice. Pathological changes in the liver including hepatocellular dysplasia were observed in aged Tg mice, suggesting that the presence of WHV transgenes may lead to liver diseases. Interestingly, Tg mice of the lineage 1281 spontaneously developed T- and B-cell responses to WHV core protein (WHcAg). DNA vaccination induced specific immune responses to WHV proteins in WHV Tg mice, indicating the tolerance break. The magnitude of the induced WHcAg-specific immune responses was dependent on the effectiveness of different DNA vaccines and was associated with a decrease in WHV loads in mice. In conclusion, sex- and age-dependent viral replication, development of autoimmune responses to viral antigens, pathological changes in the liver in the WHV Tg mice and the possibility of breaking immune tolerance to WHV transgenes will allow future studies on pathogenesis related to hepadnaviral infection and therapeutic vaccines.

Key words: Hepatitis B virus; woodchuck hepatitis virus; transgenic mouse, autoimmunity, therapeutic vaccination
Introduction

Hepatitis B virus (HBV) is a major cause of acute and chronic hepatitis in humans. The currently available treatments for hepatitis B, such as interferon-α (IFN-α) or nucleoside/nucleotide analogues are costly and have limited long-term efficacy (1, 2).

HBV has a very narrow host range and can only infect human and higher primates such as chimpanzees (3, 4). However, experiments using chimpanzees are costly and require both scientific and ethical justification. HBV-transgenic (Tg) mice have allowed examination of the influence of viral and host factors on HBV pathogenesis and replication, and assessment of the antiviral potential of pharmacological agents (5). For example, transfer of HBsAg-specific CD8+ T-cells into these mice led to the inhibition of HBV replication in the liver by a noncytolytic mechanism (6), leading to the important hypothesis that the antiviral cytokines IFN-γ and tumor necrosis factor-α are major mediators of noncytolytic inhibition of HBV replication (7). Furthermore, activation of innate immune responses in HBV Tg mice by toll-like receptor (TLR) ligands also inhibits HBV replication (8). Thus, the HBV Tg mouse model was and remains a useful research tool to study HBV infection. However, as HBV Tg mice are immunologically tolerant to HBV proteins (5, 9), obvious disease-related phenotypes have not been observed. On the other hand, HBsAg is supposed to play an important role in HBV persistence and hepatocarcinogenesis (10). The presence of HBsAg may inhibit host immune responses and facilitate HBV persistence (11, 12). Therefore, transgenic mice replicating HBV but lacking small HBs expression represent an interesting model for the studies of the role of HBsAg in HBV persistence, hepatocarcinogenesis, and antiviral immune responses (13).
Woodchuck hepatitis virus (WHV) is a member of the *Hepadnaviridae* family and was discovered in 1978 (14). WHV causes acute and chronic infections in woodchucks (*Marmota monax*), similar to HBV infections in humans. The woodchuck model has been proven to be an informative model to study hepadnaviral infection, pathogenesis, and for the screening and development of antiviral drugs (15). Despite the establishment of immunological assays and tools for the woodchuck model during the last years, immunological studies in outbred and immunogenetically uncharacterized woodchucks are still difficult (16, 17). Therefore, it was desirable to have a WHV Tg mouse model that would allow the determination of immunological parameters and complement infection experiments in woodchucks.

In the present study, we report the establishment of two WHV Tg mouse strains, one with a wild-type WHV genome and the other with a WHV genome harboring artificially introduced stop codons in the coding region of WHV surface antigen (WHsAg). The presence of WHV transgenes resulted in liver-specific but sex- and age-dependent WHV replication in Tg mice. One particularly interesting feature of the WHV Tg mice was the development of autoimmune T-cell responses to WHV core antigen and pathological changes in aged Tg mice, allowing the study of liver diseases related to hepadnaviral infection. In addition, immunizations using different DNA vaccines induced specific immune responses to WHV proteins that were associated with a decrease in WHV loads. WHV Tg mouse strains appear to be excellent tools for testing vaccines for therapy of chronic hepadnavirus infections.
Materials and methods

Molecular cloning of the transgene construct for Tg mouse generation. To construct a 1.3-fold overlength WHV genome in which pre-core and core transcription would be under the control of their endogenous promoters and enhancers, the plasmid puc119WHV-1.3 containing 1.3-fold WHV DNA (WHV nt1050-nt3320-nt2190) was constructed based on the plasmid puc119CMVWHV82 (18). In addition, a WHsAg-minus variant of WHV genome was constructed by the recombinant PCR mutagenesis method. Three stop codons were introduced into WHsAg ORF at the amino acid positions 12, 18, and 19, downstream of the WHsAg initiation codon, leading to the truncation of L-, M- and S-WHsAg. The amino acid sequence of the WHV polymerase remained intact. The wild type and WHsAg-minus WHV constructs were sequenced to exclude the presence of undesired mutations. The WHV constructs were linearized by KpnI and PvuII digestion, and then purified by gel electrophoresis prior to microinjection. C57BL/6 x C3H mice were used to make Tg mice, which were then backcrossed >10 times with C57BL/6. All mice were kept in the Central Animal Laboratory of the University of Essen. Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the local Animal Care and Use Committee of the district government (Düsseldorf, Germany) and the Fox Chase Cancer Center.

Genotyping of WHV Tg mice. Genomic DNA was extracted from the tail tissue of mice. One µg DNA of the samples was subjected to PCR to analyze for the presence of the WHV transgene. Primer pairs used for amplification of the WHV core region were: wc1, 5'- TGG...
Analysis of transgenic copy number and integration sites. The transgene copy number and number of integration sites in WHV Tg mice were determined according to the protocol published online by Thom Saunders (http://www.med.umich.edu/tamc/std.pdf). Briefly, 10 μg of hepatic genomic DNA was digested by EcoRI, phenol extracted, and precipitated, and then analyzed by Southern blot with copy standards.

Detection of WHV DNA in serum. Serum WHV DNA was extracted using the QIAamp DNA Mini Kit, and detected by real-time PCR as described previously (15), with the primers wc1 and wc-149s.

Isolation and analysis of viral RNA and WHV replicative intermediates from liver tissue. Total RNA was extracted from liver tissue samples with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Real-time reverse transcription (RT)-PCR was carried out according to the published protocols (20). Encapsidated WHV DNA was analyzed as described previously (9). WHV DNA were detected by hybridization with a 32P-labeled full-length WHV probe.

Histological examination of liver tissues in WHV Tg mice. Liver tissue samples were fixed in 10% zinc-buffered formalin (Anatek, Battle Creek, Mich.), embedded in paraffin, and
sections were cut for histological and microscopic examination according to standard methods, as described previously (21).

ELISA for detection of antibodies to WHcAg (anti-WHc) and WHsAg (anti-WHs). Anti-WHc IgGs were detected by an enzyme-linked immunosorbent assay (ELISA) as described previously (22). Total bound mouse IgG, IgG1, or IgG2a antibodies were detected with appropriate secondary antibodies labeled with horseradish peroxidase (DB Biosciences, CA) at a dilution of 1:1,000. This ELISA format did not strictly differentiate antibodies against WHcAg or WHeAg, as the preparation of recombinant WHcAg contained a fraction of polypeptides exposing various epitopes. However, the ELISA allowed the differentiation of subtypes of WHcAg/WHeAg-specific antibodies. Anti-WHs antibodies in sera were also detected by ELISA, as described previously (23). Normal C57BL/6 mouse serum samples were used as negative controls. The cut off value of ELISAs was set as 2.1 times over negative controls.

Immunization of mice by intramuscular injection of pWHcIm, pWHsIm, and pCGWHc. Plasmids pWHsIm and pWHcIm containing the WHVpreS2-S or core gene, respectively, under the control of the cytomegalovirus (CMV) promoter, were described previously (23). Plasmid pCGWHc containing WHV core gene downstream of the β-globin intron sequence, under the control of CMV promoter, was described by Kosinska et al. (24). Immunization of mice was performed according to the procedure described by Schirmbeck et al (25). Plasmid injections were repeated twice at 2-week intervals. Mice were sacrificed 2 weeks after the last immunization.

Detection of cytotoxic T lymphocytes (CTLs) by flow cytometry. WHcAg-specific CTLs
were detected by flow cytometric analysis (16). Briefly, a set of 36 synthetic peptides covering the complete WHcAg sequence, with a length of 188 amino acid residues (aa), was purchased from EMC microcollections (Tübingen, Germany). The sequences of the peptides used for the immunological assays matched the sequence of the WHV transgene. The 15-mer peptides overlapped by 10 aa and were combined in six pools (16). Three peptides containing CD8 dominant epitopes of WHcAg identified in C57BL/6 mice were used to detect the specific CTLs post immunization: aa 13-21 (YQLNFLPL), aa 6-20 (YKEFGSSYQLNLFP), and aa 11-25 (SSYQLNFLPLDFFP). An unrelated cytomegalovirus (CMV)-derived peptide YILEETSVM served as an unrelated control. For dimer staining of WHc-specific CTLs, Recombinant Soluble Dimeric Mouse H-2D[b]: Ig (DimerX, BD Bioscience) was loaded by WHc peptide aa13-21 over night, and then used to stain the splenocytes according to the technical instruction. For the determination of the frequencies of specific CD8+IFN-γ+ T cells, the cut off value was set as 2 times over the value obtained by stimulation with CMV peptide.

Up to 1×10⁶ splenocytes per well were plated in 96-well plates (Greiner Bio-One, Frickenhausen, Germany) in a total of 200 µl AIM-V medium (Gibco Invitrogen) supplemented with 10% fetal calf serum and 10 U/ml penicillin-streptomycin (PAA Laboratories, Pasching, Austria). For stimulation, peptide pools or individual peptides were added to a final concentration of 2 µg/ml. After 3 days, a final concentration of 10 U/ml recombinant human interleukin 2 (Roche Diagnostics, Mannheim, Germany) was added. Flow cytometric analysis was carried out with a FACSCalibur flow cytometer (Becton Dickinson, Heidelberg, Germany). In all cases, at least 100,000 events were collected for each
sample. Data files were analyzed with FlowJo software (Tree Star, Ashland, Oregon).
Results

Genomic features of Tg mice. WHV Tg mice with the wild-type WHV genome and mutated version unable to express WHsAg were generated (Figure 1A) and designated as lineages 1217 and 1281, respectively. Integrated WHV DNA and flanking nucleotide sequence were analyzed by IPCR. Sequence analysis of WHV transgenes in the mouse genome excluded unexpected mutations in the integrated WHV sequences (data not shown). IPCR generated a single PCR product each. Direct sequencing of PCR products suggested that only a single integration site of WHV genome in the mouse genome in both WHV Tg mouse strains (data not shown). The integration sites of the transgenes were mapped, showing that WHV transgenes were integrated into chromosome 10 of lineage 1217 mice and into the chromosome 5 of lineage 1281 mice (data not shown). Southern blot analysis of EcoRI-restricted genomic DNA of WHV Tg mice detected only 2 bands when a complete WHV genome was used as probe. The signals were comparable with that of a single copy WHV standard, indicating that a single copy of the WHV genome was integrated at a single site in the mouse genome of both lineages 1217 and 1281 (Figure 1B), consistently with the cloning results.

WHV gene expression and replication in the Tg mice are liver-specific, age- and sex-dependent. The levels of WHV replicative intermediates were significantly lower in the liver of mice of lineage 1217 compared to those of lineage 1281 (Figure 2A). Encapsidated WHV replicative intermediates were detected only in the livers but not in kidney and spleen from both lineages of WHV Tg mice (Figure 2B). These bands were comparable to the
signals detected in liver tissues from WHV chronically infected woodchucks. Interestingly, WHV replication increased in an age- and sex- dependent manner. WHV replication intermediates were detected at 2 weeks of age and increased as the mice reached maturity, with high levels being detected through 40 weeks of age (Figures 2B, 2C). Compared with female mice, male mice had higher levels of WHV replicative intermediates in the liver (Figure 2C) and significantly higher levels of serum WHV DNA loads (Figure 2D).

The 3.7-kb pregenome and 2.1-kb mRNA transcripts were detectable in the liver, equally in males and females of both lineages (data not shown). The levels of WHV mRNA in mice of lineage 1281 were relative stable and age- and sex-independent (Figure 2E). The 0.7-kb viral mRNA corresponding to the WHV x gene transcript was not detected by Northern blot analysis in any sample from these animals (data not shown). This finding is consistent with the low-level of expression of the WHV x-gene in the woodchuck liver during natural infection (26). The detection of WHV proteins failed so far, as no suitable antibodies were available (15, 24, 27, 28).

Serum WHV DNA was only detected in mice of lineage 1217 with the wild type WHV transgene (Figure 2D) but not in that of lineage 1281 with WHsAg stop codon mutation (data not shown). This result is consistent with the fact that the formation of secreted WHV virions requires intact WHsAg.

**Age-dependent development of hepatocellular dysplasia in WHV Tg mice.** Liver from 99 WHV Tg mice was examined for histological changes. In both WHV Tg lineages, animals younger than 11 W (n = 80, 25 1217-mice, 14 male and 11 female; 55 1281-mice, 31 male and 24 female) showed normal or slight non-specific hepatocellular damage. However, older
animals, between 37 weeks and 41 weeks of age (n = 19, 10 1217-mice, 4 male and 6 female; 9 1281-mice, 6 male and 3 female), all exhibited dysplasia, with increased numbers of double nucleated hepatocytes, variation in the size of hepatocellular nuclei, and variation in the cellular density of hepatocytes (Figure 3). No differences were observed between male and female Tg mice in the development of dysplastic changes. Interestingly, 1 of the 10 aged 1217 mice (male, age 40 w) developed HCC.

Spontaneous development of humoral and cellular immune responses to WHV proteins.

To investigate whether the WHV transgene products WHsAg and WHcAg are recognized by the host immune system, antibodies to WHsAg and WHcAg, and CTLs to WHcAg epitopes were determined in WHV Tg mice. All WHV Tg mice were negative for anti-WHs antibodies. However, anti-WHc antibodies were detectable in mice of both lineages, starting at 9 weeks of age. Interestingly, the development of anti-WHc antibodies increased in age-dependent manner in mice of the lineage 1281 (Figure 4A). More than 50% of the mice older than 16 weeks of age showed high titers of serum anti-WHc antibodies. Moreover, more male mice developed anti-WHc antibodies than females at any age (Figure 4A; Table 1). In mice of the lineage 1217, anti-WHc antibody was detectable in about 60% of mice (Figure 4C; Table 1), and there was no significant difference in the titers of serum anti-WHc antibodies between male and female 1217 mice (Figure 4D).

IFN-γ secreting WHc-specific CTLs were detectable at very low levels in young mice of both WHV Tg lineages only when splenocytes were stimulated with WHcAg peptide pool 1 (Figure 4E, upper panel). Consistent with the levels of anti-WHc antibodies, WHcAg-specific CTLs were more frequently detected in mice of the lineage 1281 compared with lineage 1217.
DNA vaccination induced WHV-specific immune responses and led to the suppression of WHV replication in WHV Tg mice. WHV Tg mice at 8-10 weeks of age were tested for the presence of anti-WHc antibodies. Mice negative for anti-WHc were selected to test the immune response to DNA immunization. Immunization with pWHsIm induced anti-WHs antibodies in mice of the lineage 1281 but not the lineage 1217 (data not shown). This is consistent with the fact that the wild type WHV transgene is able to produce WHsAg and may cause tolerance to WHsAg. Immunization with pWHcIm induced both anti-WHc antibodies and WHcAg-specific CTLs in mice of both lineages (Figure 5A-C). However, the immune responses were significantly weaker than those in non-transgenic C57BL/6 mice that underwent the same immunization protocol (Figure 5A, and 5B). WHcAg-specific T cell responses in Tg mice were weak and only detectable after the in vitro expansion of specific T-cells with peptides for 7 days (Figure 5B and C). In contrast to the spontaneous cellular response to WHcAg, WHcAg vaccination-induced CTLs in the Tg mice recognized the dominant epitope WHcAg aa 6-20, as also seen in C57BL/6 mice immunized with WHcAg.
(Figure 5C). However, only part of the specific CD8 cells maintained the ability to produce IFN-\(\gamma\) or degranulate after stimulation with the WHcAg peptide aa 6-20, which indicated that the WHcAg-specific CD8 cells were functionally impaired. Interestingly, pWHcIm immunization resulted in a significant decrease of serum viral load (Figure 5D), even though the vaccination-induced CD8 cell response was weak.

Further, pCGWHc, a vector with a higher WHcAg expression, was tested in the WHV Tg mice. Interestingly, pCGWHc immunizations induced significantly higher levels of anti-WHc IgG2a antibodies and IFN-\(\gamma\) producing WHcAg-specific CD8 cells in mice of the lineage 1217 (Figure 5E-G) and also led to significant decrease of serum viral loads (Figure 5H).
Discussion

In this study, two Tg mouse strains with 1.3-fold over-length WHV genomes were established. These WHV Tg mouse strains revealed liver-specific, age- and sex-dependent viral replication activity and developed spontaneously humoral and cellular immune responses to WHV antigens with a high frequency. In addition, aged mice of these strains showed pathological changes in the liver. Thus, these novel WHV Tg mice represent useful models to study clinically relevant features related to hepadnaviral infections.

The low levels of viral gene expression and replication in the liver of 1217 lineage mice with the wild-type WHV genome are consistent with results observed in HBV Tg mice (21, 29). Halverscheid et al (13) observed high levels of viral gene expression and replication in the liver of HBV Tg mice lacking expression of small HBs. In the present study, significantly higher levels of viral replication were observed in the liver of mice of the lineage 1281, which harbor the S minus WHV genome construct compared to the lineage 1217. Accumulation of core antigen/particles in the hepatocytes may contribute to high level of viral replication in the liver of Tg mice lacking expression of envelope proteins. No viral particles were found in the serum of mice of the lineage 1281, as the mutated WHV genome was not able to express WHsAg.

WHV replication appeared higher in males than females in both lineages of WHV Tg mice. The same phenomenon was also observed in HBV Tg mice (13, 30), consistent with HBV infections in humans. The surrounding sequences of WHV transgenes integration sites in the mouse genome of lineages 1217 and 1281 are referred to AC123865.5 and AC159977.4,
respectively (data not shown) and contain repeated sequences with some undefined regions.

No functional genes were identified in the direct neighborhood of WHV transgenes within the ranges of 3 to 10 kb, making unlikely that the sex- and tissue-specific WHV replication was determined by such specific host genes. Certainly, the insertion of WHV transgenes may potentially cause chromosome abnormality and this possibility could not be completely ruled out. Higher levels of WHV replicative intermediates in male than female animals suggests that the male androgen axis regulates HBV replication (31). It is reported by different research groups that male patients with chronic HBV infections have higher viral loads and tend to develop HCC with a significantly higher frequency than female patients (~5-11:1) (32, 33). The consistent male predominance in liver injury and incidence of HCC in both human and in rodents in the context of hepadnaviral infection supports the concept that sex is an important factor with respect to hepatocarcinogenesis in general.

One interesting feature of the WHV Tg mouse strains is the development of autoimmune CTL-responses to WHV proteins with the age. Higher levels of IgG2a antibodies against WHcAg were detectable in the sera of mice of the lineage 1281 compared to the lineage 1217. This could be explained by the fact that the intracellular retention of WHcAg in hepatocytes in mice of the lineage 1281 leads to accumulation of WHcAg in situ, thus increasing the possibility of being presented to immune cells. It is also possible that WHcAg is released into peripheral blood by dead hepatocytes and induces specific antibody responses. WHcAg-specific CTLs were detected in 6 of 28 mice of lineage 1281, indicating that WHcAg induced weak cellular immune responses in WHV Tg mice. The different levels of T cell exhaustion, circulating WHV DNA and/or antigenemia may contribute to the difference in
responses to the WHV transgenes. The cellular responses were not strong enough to suppress
WHV replication, but might contribute to the nonspecific infiltration of inflammatory cells
into the liver. There was no obvious difference between mice with and without detectable
immune responses in terms of the levels of liver pathology, serum WHV DNA, and
intrahepatic WHV replication intermediates.

There was no WHV-specific hepatocellular damage in the livers of young WHV Tg mice,
consistent with the general notion that the pathogenesis of chronic HBV infection is
immune-mediated (34). The observed slight unspecific hepatocellular damage, lobular
nodular infiltrates of inflammatory cells might have been caused by the weak immune
responses to the WHV Tg products. Interestingly, dysplasia developed in all aged Tg mice,
and hepatocellular carcinoma was found in one WHV Tg mouse, providing more supporting
evidence that hepadnaviruses, including WHV, are carcinogenic (35, 36). In addition, the
development of immune responses may lead to liver injury over an extended period of time.
This issue remains to be investigated in the future.

The induction of WHsAg-specific antibodies occurred in mice of the lineage 1281 but not in
the lineage 1217 mice following vaccination with pWHsIm, suggesting that mice of the
lineage 1217 are tolerant to WHsAg due to the constitutive expression beginning early in life.
Two plasmids expressing WHcAg, pWHeIm and pCGWHe, induced WHcAg-specific T cell
responses, indicating that it is possible to break WHV-specific immune tolerance. DNA
vaccines-induced CTLs and reduced serum WHV DNA loads to a low level of $10^3$ to $10^4$
copies/ml. The possibility of breaking immune tolerance to WHcAg in Tg mice allows the
testing of different therapeutic vaccines and vaccination regimens. Recently, an immunization
protocol including priming with DNA vaccines and boosts with adenoviral vectors was shown
to elicit robust and effective humoral and cellular immune response against WHV in
woodchucks (24, 37). This protocol was also evaluated in the Tg mice and was superior to the
DNA vaccination alone (Kosinska et al., unpublished data). Thus, the WHV Tg mouse stains
may be valuable research tools for the development of therapeutic vaccines against chronic
HBV infection. Taken together, WHV Tg mice represent new animal models for the study of
hepadnaviral infection and pathogenesis related to viral hepatitis and will be especially useful
in the development of new antiviral strategies.
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References:


Figure legends

**Figure 1: Generation and identification of WHV Tg mice.** (A) Structure of plasmid puc119WHV-1.3. Schematic representation of 1.3-fold greater-than-genome WHV constructs for the generation of WHV Tg mice. En, enhancer; Poly A, polyadenylation signal; The black ovals indicate the promoter sequences of WHV ORFs. The three stop codons introduced into the S ORF are underlined. (B) The transgenic copy number and the number of integration sites of WHV transgenes. A total of 10 µg of mouse genomic DNA was digested with EcoRI and then subjected to Southern blot analysis with linear full-length WHV copy standards.

**Figure 2: Organ-, Age-, and Sex-dependent WHV gene expression and replication in Tg mice.** (A-C) WHV replicative intermediates in the liver, spleen, and kidney of mice of lineage 1281 (A-C) and 1217 (A) were analyzed by Southern blotting. (D) WHV viral loads in the serum of mice of lineage 1217 were determined by real-time PCR. (E) Intrahepatic WHV mRNAs in mice of lineage 1281 were determined by real-time RT-PCR. PWHs, primary woodchuck hepatocytes; M, male; F, female; L, liver; K, kidney; S, spleen; ET, exposure time.

**Figure 3: Histological investigation of liver tissues.** Liver tissue samples were fixed in formalin and embedded in paraffin. Sections were cut and processed for histological and microscopic examination according to standard methods. Representative photos were shown for the histological presentation of normal and dysplastic changes of liver sections. The characteristically histological presentation of HCC found in an aged mouse of lineage 1217 was also shown. Arrow show inflammatory cell infiltrates in the H&E stained section.
Figure 4: WHcAg-specific humoral and cellular immune response in WHV Tg mice. Anti-WHc antibodies in the serum of WHV Tg mice of lineages 1281 (A, B) and 1217 (C, D) were detected by ELISA. WHcAg-specific CTLs (E) in the splenocytes of Tg mice of lineage 1281 were analyzed by flow cytometry. The mice T88 and T164 of lineage 1281 were two representative examples positive or negative for WHcAg-specific CTL responses, respectively.

Figure 5: Immunization-induced humoral and cellular responses to WHcAg in WHV Tg mice. WHV Tg mice of lineage 1217 (wild type WHV) and normal C57BL/6 mice were immunized three times with pWHcIm (A-D) or pCGWHc (E-H). Serum anti-WHc antibodies (A and E), WHcAg-specific CTLs after in vitro expansion in the presence of specific peptides for 7 days (B, C), or ex vivo stimulation for 4 hours (F, G), and serum WHV DNA loads (D and H) were determined by ELISA, flow cytometry, and real-time PCR, respectively.
Table 1. Occurrence of anti-WHc antibodies and WHcAg-specific CTL in WHV Tg mice

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