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Break of T Cell Ignorance to a Viral Antigen in the Liver Induces Hepatitis

David Voehringer,* Claudine Blaser,† Andrea Busse Grawitz,‡ Francis V. Chisari,† Kurt Buerki,* and Hanspeter Pircher²*

To study peripheral tolerance of CD8 T cells to a classically MHC-restricted peptide Ag expressed in hepatocytes, ALB1 transgenic (tg) mice expressing the CTL epitope GP33 of the lymphocytic choriomeningitis virus glycoprotein under control of the mouse albumin promoter were generated. ALB1 mice exclusively expressed the GP33 transgene in the liver and, at a 100- to 1000-fold lower level, in the thymus. TCR-tg mice specific for the GP33 epitope were used to directly follow GP33-specific T cells in vivo. These experiments revealed that 1) thymic expression of the GP33 transgene led to incomplete central deletion of TCR-tg cells; and 2) peripheral TCR-tg cells in ALB1 mice ignored the GP33 transgene expressed in hepatocytes. Ignorance of adaptively transferred TCR-tg cells in ALB1 mice was broken by infection with lymphocytic choriomeningitis virus, leading to induction of hepatitis in ALB1, but not in control, mice. Taken together, we have established a novel model of virus-induced CD8 T cell-mediated autoimmune hepatitis in mice and demonstrate that naive CD8 T cells may ignore Ags expressed in the liver. The Journal of Immunology, 2000, 165: 2415–2422.

Mechanisms of tolerance and their implications for autoimmune diseases are of central interest in immunology. In the past few years considerable progress in our understanding of the basic mechanisms of immunological tolerance induction has been made using transgenic mouse models. However, we do not yet understand all the factors involved in this process. T cell maturation takes place in the thymus, where T cells are positively selected for their ability to recognize self-MHC molecules and are negatively selected if the avidity of Ag recognition is too high. This produces a peripheral T cell repertoire that is not reactive against self-Ags present in the thymus. In addition, APC in the thymus can present a variety of ectopically expressed peripheral Ags to maturing T cells (1–3) and soluble Ags can reach the thymus and be presented on bone marrow-derived APC (4, 5). However, the thymus may not present all self-Ags, and therefore peripheral tolerance mechanisms are also required. Peripheral deletion (6–8), induction of anergy (9–12), and ignorance (13, 14) have all been demonstrated to lead to peripheral T cell tolerance.

The accessibility of peripheral organs for T cells may play a crucial role in tolerance induction. Blood vessels in most organs are surrounded by a basement membrane, restricting tissue access of T cells. The liver is an exception in this respect, as it lacks a basement membrane around hepatic sinusoids. Therefore, hepatocytes are readily accessible for T cells (15). Thus, it is likely that active mechanisms of tolerance induction, such as peripheral deletion or anergy, are operating in the liver. Several earlier studies have examined CD8 T cell tolerance induction by expression of specific Ags in the liver (16–18). However, the tolerance mechanisms were not well defined, since the fate of self-reactive T cells could not be directly followed. Surprisingly, tolerance mechanisms of CD8 T cells toward liver Ags using a transgene-encoded neo self Ag together with the corresponding TCR-transgenic (tg)³ models have to date only been studied with the allo-MHC Ag Kb (19–22). These studies have defined peripheral deletion and anergy as the key mechanisms of tolerance induction of CD8 T cells in the liver.

To examine the tolerance mechanism of CD8 T cells toward a classical MHC-restricted peptide Ag in the liver, we have generated tg mice expressing the GP33 CD8 T cell epitope of LCMV glycoprotein in hepatocytes. These mice together with TCR-tg mice specific for the GP33 epitope were used to study peripheral tolerance of CD8 T cells toward an MHC-restricted peptide Ag in the liver.

Materials and Methods

Mice

The DNA construct used to generate ALB1 mice was based on the pWEKB plasmid containing the genomic K b gene in which the 5’ part of exon 1 was replaced by a sDNA fragment encoding aa 1–60 of the LCMV glycoprotein (23). The NspI/EcoRI fragment of pWEKB containing the GP33-modified K b gene without the promoter region was cloned into the EcoRI/SmaI site of pUC-19. The 3.8-kb fragment of the 5’ noncoding region of the mouse albumin gene containing promoter and enhancer elements (24) was cloned in front of the GP33-modified K b gene. The hybrid construct was released from the plasmid by a SpeI and PvuII digest, leaving 100 bp of vector sequence at the 5’ end of the regulatory elements. DNA was injected into mouse zygotes of C57BL/6 × DBA/2F2 mice. Six founder mice were obtained, and one line (ALB1) was selected for further analysis. The mice used in this study were back-crossed to C57BL/6 (B6) eight or nine times. B6 mice were obtained from our own breeding colony or from Charles River (Sulzfeld, Germany). TCR-tg mice (line 318) expressing the CTL epitope GP33 of the lymphocytic choriomeningitis virus glycoprotein under control of the mouse albumin promoter were generated. ALB1 mice exclusively expressed the GP33 transgene in the liver and, at a 100- to 1000-fold lower level, in the thymus. TCR-tg mice specific for the GP33 epitope were used to directly follow GP33-specific T cells in vivo. These experiments revealed that 1) thymic expression of the GP33 transgene led to incomplete central deletion of TCR-tg cells; and 2) peripheral TCR-tg cells in ALB1 mice ignored the GP33 transgene expressed in hepatocytes. Ignorance of adaptively transferred TCR-tg cells in ALB1 mice was broken by infection with lymphocytic choriomeningitis virus, leading to induction of hepatitis in ALB1, but not in control, mice. Taken together, we have established a novel model of virus-induced CD8 T cell-mediated autoimmune hepatitis in mice and demonstrate that naive CD8 T cells may ignore Ags expressed in the liver. The Journal of Immunology, 2000, 165: 2415–2422.

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3 Abbreviations used in this paper: tg, transgenic; B6, C57BL/6; LCMV, lymphocytic choriomeningitis virus; HBsAg, hepatitis B virus large surface Ag; CFSE, 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester; sALT, serum alanine aminotransferase; SP, single positive.
75% of their CD8+ T cells have been described previously (26). To generate TCR-tg Thy-1.1 mice, 318 mice were back-crossed to B6.PL-Thy-1+ mice, which were a gift from Dr. H. Mossmann (Max Planck Institute for Immunobiology, Freiburg, Germany).

107.5 g mice (official designation Tg[Alb-1.HBV][Bri66]) expressing hepatitis B virus large surface antigen (HBsAg) in the liver have been described (27). They were back-crossed three times to B6 and MHC typed before use. The tg mice were typed by PCR using 5′-gPTY (5′-CCAGTAAAGAGAT GGGTCAG-3′) and 3′ gPTY (5′-GGGTCGATGACGGCCGG-3′) primers for the LCMV glycoprotein in the liver were generated. Our first attempts to express the GP33 Ag in hepatocytes using the entire LCMV glycoprotein cDNA under the control of the mouse albumin promoter failed (data not shown). For a second approach, a chimeric albumin-GP33-Kb construct was designed. In this construct the Kb promoter region of the genomic Kb gene was replaced by the mouse albumin promoter, and the signal sequence of the Kb molecule, encoded in the first exon, was exchanged by the leader sequence of the LCMV glycoprotein (aa 1–60) containing the GP33 epitope (Fig. 1A). Six tg lines were obtained that showed similar phenotypes, and one line (ALB1 mice) was selected for further analysis. RT-PCR analysis of this line revealed GP33 transgene expression in hepatocyte lines, and one line (ALB1 mice) was selected for further analysis. RT-PCR analysis of this line revealed GP33 transgene expression in hepatocytes from TCR-tg mice induced a specific increase in transaminase levels. As shown in Fig. 1B, expression of the GP33 transgene significantly increased in the liver and the thymus (Fig. 1B), but not in other organs, including heart, kidney, lung, and spleen (data not shown). Importantly, as illustrated in Fig. 1B, expression of the GP33 transgene in the liver was 100- to 1000-fold higher than that in the thymus.

To examine whether the GP33 transgene was sufficiently expressed in hepatocytes to be recognized by GP33-specific T cells, activated CD8 T cells from LCMV TCR-tg mice (line 318) specific for the GP33 epitope (26) were transferred into nonirradiated ALB1 mice and transaminase (sALT) levels in the sera were monitored. As shown in Fig. 1C, adoptively transferred effector cells from TCR-tg mice induced a specific increase in transaminase levels in ALB1, but not in B6 control mice, indicating that the GP33 transgene on hepatocytes could be recognized by GP33-specific effector T cells.

**Molecular biology**

Total RNA was isolated using an RNA isolation kit (Fluka BioChemika, Buchs, Switzerland). For cDNA synthesis 2 μg of total RNA was reverse transcribed using the Superscript II reverse transcriptase (Life Technologies, Gaithersburg, MD). RT-PCR was performed by amplification of cDNA with GP33 transgene-specific primers 5′-gPTY (5′-CCAGTAAAGAGAT GGGTCAG-3′) and 3′ gPTY (5′-GGGTCGATGACGGCCGG-3′) primers for ALB1 mice and HBS93 (5′-gPTY). Genes were labeled with 5′-CCAGATGCGATGCGG-3′ and HBS539 (5′-GGGTCGATGACGGCCGG-3′) primers for 107.5 g mice.

**Results**

**LCMV GP33 expression in ALB1-tg mice**

To study T cell tolerance toward an Ag expressed in hepatocytes, tg mice expressing the GP33 CD8 T cell epitope of LCMV glycoprotein were adoptively transferred into naive TCR-tg Thy-1.1 mice (line 318) into B6.PL-Thy-1+ mice, indicating that the GP33 transgene did not lead to complete tolerance induction. However, the GP33-specific CTL activity of naive TCR-tg Thy-1.1 mice, adoptively transferred effector cells from TCR-tg mice induced a specific increase in transaminase levels in ALB1, but not in B6 control mice, indicating that the GP33 transgene on hepatocytes could be recognized by GP33-specific effector T cells.

**Incomplete thymic deletion of GP33-specific T cells in ALB1 mice**

To examine the effect of the GP33 transgene on the T cell repertoire, ALB1 mice were infected with LCMV, and the Ag specificity of the induced CTL response was tested. As shown in Fig. 2A, spleen cells from LCMV-infected ALB1 mice exhibited significant CTL activity against GP33 peptide-loaded EL-4 target cells, indicating that the GP33 transgene did not lead to complete tolerance induction. However, the GP33-specific CTL activity of ALB1 mice was reduced about 10-fold on an effector cell-to-target basis compared with that of C57BL/6 (B6) control mice. This partial tolerance was GP33 Ag specific, since LCMV-infected ALB1 mice showed the same CTL activity against LCMV NP396 peptide-coated target cells as B6 mice (Fig. 2B). Titration experiments further revealed a more pronounced decrease in specific lysis at low GP33 peptide concentration by ALB1 effector cells compared with LCMV-infected B6 control mice. This partial tolerance was GP33 Ag specific, since LCMV-infected ALB1 mice showed the same CTL activity against LCMV NP396 peptide-coated target cells as B6 mice (Fig. 2B). Titration experiments further revealed a more pronounced decrease in specific lysis at low GP33 peptide concentration by ALB1 effector cells compared with LCMV-infected B6 control mice.
To examine the mechanism of tolerance induction, ALB1 mice were mated with LCMV GP33 TCR-tg mice, and the number of GP33-specific TCR-tg cells (Vα2⁺/Vβ8⁺) in the thymus and the periphery were analyzed by flow cytometry (Fig. 3). The size of the thymus and the total number of thymocytes were similar in TCR × ALB1, TCR, and ALB1 mice. Four-color analysis showed a reduction of CD8 single-positive (SP) thymocytes expressing the tg TCR (7.2%) in TCR × ALB1 mice compared with TCR mice (37%).

In the periphery of TCR × ALB1 double-tg mice, the percentage of CD8 T cells expressing the tg TCR (4.5%) was decreased about 8- to 10-fold compared with that of single-tg TCR mice (39%). Importantly, the percentage of Vα2⁺/Vβ8⁺ T cells in these mice was still significantly higher than the number of T cells expressing only endogenous TCR Vα2 and Vβ8 chains in control mice (2%). This clearly showed that TCR-tg cells were present in the periphery of TCR × ALB1 double-tg mice. To test whether the remaining TCR-tg cells in TCR × ALB1 mice were functional, TCR-tg cells from TCR and TCR × ALB1 mice were stimulated for 4 days with GP33-coated APC in vitro in the absence of exogenous IL-2, and the cytolytic activity was assessed. To generate identical conditions for the stimulation, normal B6 spleen cells were added to the responder spleen cells from TCR mice to ensure the same percentage (~0.5%) of TCR-tg cells in the culture. As shown in Fig. 4, the GP33-specific lytic activity of the cultures from TCR and TCR × ALB1 mice were comparable, indicating that the TCR-tg cells from TCR × ALB1 mice were not anergic. Taken together, these data indicate that thymic expression of the GP33 transgene resulted in clonal deletion of about 90% of GP33-specific T cells. However, 10% of the cells escaped tolerance induction in the thymus, migrated to the periphery, and remained functional.

Adaptively transferred naive TCR-tg T cells ignore the GP33 Ag expressed in the liver of ALB1 mice

To determine whether TCR-tg cells proliferate in response to the GP33 Ag in the liver of ALB1 mice, we made use of the CFSE labeling technique (28). Naive CFSE-labeled TCR-tg Thy-1.1⁺ T cells (10⁷) were adoptively transferred into nonirradiated ALB1 mice (Thy-1.2), and 4 days later TCR-tg cells isolated from the liver were analyzed. The data in Fig. 5 demonstrate that TCR-tg Thy-1.1⁺ cells isolated from liver homogenate of ALB1 mice did not undergo cell division, whereas the same TCR-tg cells transfused into LCMV-infected hosts cycled rapidly.

The lack of reactivity of naive TCR-tg cells to the GP33 expressed in hepatocytes may be due to an inefficient traffic of these cells through the parenchyma of the liver. To address this issue, 10⁷ naive or activated TCR-tg cells (Thy-1.1⁺) were transferred into ALB1 mice, and after 18 h the localization of these cells in the liver was monitored by immunohistology. As shown in Fig. 6A, naive TCR-tg cells were rarely found in the liver, whereas activated TCR-tg cells (Fig. 6B) efficiently infiltrated this organ. Taken together, these data indicated that in contrast to activated TCR-tg cells most of the naive TCR-tg cells did not contact GP33-expressing hepatocytes in these short-term experiments.

To examine whether expression of GP33 in the liver influenced survival or responsiveness of naive TCR-tg cells in long-term assays, naive TCR-tg cells (10⁷) were transferred into ALB1 mice, and the fate of the transferred TCR-tg cells was followed for 2 mo. As shown in Fig. 7A, the frequency of the transferred cells slowly declined in ALB1 and control B6 mice with similar kinetics. A slow decline of the transferred T cells is expected due to their

FIGURE 1. A, Schematic picture of the transgenic construct. The construct consists of the mouse albumin promoter fused to the promotorless genomic H-2Kb gene, in which the signal sequence of the Kb molecule encoded by exon 1 was replaced by the leader sequence (aa 1–60) of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct consists of the mouse albumin promoter fused to the promotorless genomic H-2Kb gene, in which the signal sequence of the Kb molecule encoded by exon 1 was replaced by the leader sequence (aa 1–60) of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the LCMV glycoprotein (GP) encoding the GP33 epitope (aa 33–41). The construct does not encode a functional Kb molecule, since insertion of the
limited lifespan and the generation of new T cells in the thymus of the recipient mice. Importantly, the frequency of TCR-tg (Vα21) cells in the donor cell population (Thy-1.1CD81) remained constant in both types of mice (Fig. 7B). After 9 wk, when the transferred cells were almost undetectable by flow cytometry, mice were infected with LCMV. The LCMV infection induced an identical clonal expansion of the transferred TCR-tg cells in both types of recipient mice (Fig. 7A). Taken together, these experiments indicated that the GP33-Ag expressed in the liver of ALB1 mice induced neither cell division, peripheral deletion, nor induction of nonresponsiveness of adoptively transferred TCR-tg cells.

The data presented above indicate that the GP33 Ag expressed on hepatocytes in ALB1 mice was ignored by adoptively transferred naive GP33-specific TCR-tg cells. To test whether these cells could be activated in vivo by LCMV infection and whether the induced T cells recognize the GP33 Ag on hepatocytes and induce hepatitis, transaminase (sALT) levels in the sera were monitored. Indeed, as shown in Fig. 8A, LCMV infection of ALB1 mice containing 10^5 naive TCR-tg cells led to a significant increase in sALT levels (200–300 U/L). Without transfer of TCR-tg cells or without LCMV infection sALT levels were <50 U/L in these mice (data not shown).

Expression of the HBsAg in the liver of the tg line 107.5 leads to an increased sensitivity of hepatocytes to inflammatory cytokines due to storage of HBsAg in the endoplasmic reticulum (27, 30). To increase the sensitivity of our model system, ALB1×107.5 double-tg mice expressing both HBsAg and GP33 Ag in hepatocytes were generated. Adoptive transfer of TCR-tg cells followed by LCMV infection did not result in an increase in sALT levels in 107.5 single-tg mice (Fig. 8B). In striking contrast, ALB1×107.5 double-tg recipient mice of TCR-tg cells developed...
hepatitis, as evidenced by high sALT levels (600–800 U/L) after LCMV infection (Fig. 8 C). These data underline the Ag specificity of this model and demonstrate that naive TCR-tg cells activated in vivo by the LCMV infection are able to recognize the GP33 Ag expressed in hepatocytes of ALB1 mice.

Surprisingly, TCR3 ALB13 107.5 triple-transgenic mice showed only weak signs of hepatitis (≤100 U/L) after LCMV infection (Fig. 8 D). This result, however, can be explained by the limited clonal expansion of TCR-tg T cells in these mice after LCMV infection compared with the extent of expansion in the adoptive transfer experiments (Fig. 8, D vs A–C). The weak proliferative response of the TCR-tg cells in TCR3 ALB13 107.5 triple-tg mice was due to rapid LCMV clearance (<10⁷ PFU/g spleen vs 10⁷ in control mice on day 4 after infection) by TCR-tg cells. To provide further support for this explanation, high numbers (10⁷) of naive TCR-tg cells were transferred into ALB13 107.5 mice followed by LCMV infection. This cell transfer resulted in a frequency of TCR-tg T cells in the recipient mice similar to that seen in TCR × ALB1 × 107.5 triple-tg mice. LCMV infection of these ALB1 × 107.5 double-tg mice also induced only moderate clonal expansion of TCR-tg cells and mild hepatitis (<150 U/L; Fig. 8E).

Finally, we investigated whether TCR-tg cells isolated from TCR × ALB1 double-tg or TCR × ALB1 × 107.5 triple-tg mice were able to proliferate and to induce hepatitis after in vivo activation. TCR-tg cells (10⁵) from these mice were transferred into ALB1 × 107.5 double-tg mice. After cell transfer, mice were infected with LCMV, and clonal expansion of the transferred TCR-tg cells and sALT levels was monitored. The same degree of expansion and hepatitis was observed with TCR-tg cells from TCR × ALB1 (Fig. 8F) or TCR × ALB1 × 107.5 mice (Fig. 8G) as seen with transfers of T cells from single-tg TCR mice (Fig. 8C). This result demonstrates that TCR-tg cells in TCR × ALB1 or TCR × ALB1 × 107.5 mice were not anergized by the presence of the GP33 Ag on hepatocytes.

**Discussion**

To study tolerance of CD8 T cells toward an Ag expressed in the liver, we generated tg mice (ALB1 mice) expressing the GP33 epitope of the LCMV glycoprotein in hepatocytes. The analysis of ALB1 mice revealed that the GP33 Ag expressed in hepatocytes is ignored by GP33-specific TCR-tg cells. Ignorance can be broken by infection with LCMV, leading to activation of TCR-tg cells and induction of hepatitis.
The albumin promoter has been used previously to achieve liver-specific expression of a transgene (31, 32). In six tg lines analyzed here, expression of the GP33 transgene was not strictly liver specific, and thymic expression was observed in all founder lines. Thymic expression of tg neo-self Ags under the control of putative tissue-specific promoters has been commonly found. In addition, ectopic expression of various endogenous genes normally expressed in the periphery has been frequently observed in the thymus (1–3). These observations suggest that thymic expression of peripheral self Ags may serve a physiological purpose in establishment of self tolerance to nonthymic proteins.

Analysis of TCR × ALB1 double-tg mice revealed that GP33 transgene expression in the thymus led to an 8- to 10-fold reduced number of TCR-tg cells in the thymus and the periphery. Importantly, the density of the tg TCR and of CD8 coreceptors on the remaining TCR-tg cells in TCR × ALB1 double-tg mice was similar to that seen on T cells from single-tg TCR mice. Furthermore, TCR-tg cells from TCR × ALB1 double-tg or TCR × ALB1 × 107.5 triple-tg mice exhibited the same capacity for clonal expansion and induction of hepatitis after adoptive transfer and LCMV infection as tg cells from single-tg TCR mice. In this respect, our system differs from other tolerance models in which the neo-self infection as tg cells from single-tg TCR mice. In this respect, our system differs from other tolerance models in which the neo-self Ag expressed on hepatocytes. This conclusion is based on the

FIGURE 8. Clonal expansion of TCR-tg cells and transaminase (sALT) levels after LCMV infection. A–C, TCR-tg cells (10⁵) were adoptively transferred into ALB1 (A), 107.5 (B), and ALB1 × 107.5 double-tg (C) mice. One day after transfer, recipient mice were infected with LCMV, and sALT levels in the sera (bars) and clonal expansion of TCR-tg cells (○) were determined at the indicated time points. Clonal expansion and sALT levels were also determined in LCMV-infected TCR × ALB1 × 107.5 triple-tg mice (D) and in infected ALB1 × 107.5 double-tg mice (E) adoptively transferred with 10⁷ TCR-tg cells. F and G, TCR-tg cells (10⁵) isolated from TCR × ALB1 double-tg (F) or TCR × ALB1 × 107.5 triple-tg (G) mice were transferred into ALB1 × 107.5 recipient mice followed by LCMV infection. In all experiments nonirradiated recipient mice were used, and the values represent means from at least three individual mice per group.

Without transfer of GP33-specific T cells, ALB1 single-tg mice did not develop disease after LCMV infection, probably because a large proportion (~90%) of GP33-specific T cells were deleted in the thymus. The finding that transfer of a small number (10⁵) of naive GP33-specific TCR-tg cells restored responsiveness and was sufficient to induce autoimmune hepatitis after LCMV infection supports this hypothesis. Furthermore, it indicates that significant transaminase levels were only observed in this system when a massive GP33-specific CTL response was induced. Interestingly, transaminase levels were increased only transiently despite the persistence of TCR-tg effector/memory cells at a high frequency at later time points. This phenomenon requires further study.

Surprisingly, LCMV infection of TCR × ALB1 × 107.5 triple-tg (Fig. 8D) or ALB1 × 107.5 double-tg mice (Fig. 8E) adoptively transferred with a high number of TCR-tg cells did not induce a significant increase in transaminase levels. How can this result be explained? Due to the high frequency of LCMV GP33-specific TCR-tg cells, LCMV is rapidly cleared under these conditions. Therefore, Ag-induced expansion of TCR-tg cells is very limited, and thus, transaminase levels are not significantly increased.

Once they had left the thymus, TCR-tg cells ignored the GP33 Ag expressed on hepatocytes. This conclusion is based on the
adoptive transfer experiments, which revealed that TCR-tg cells did not undergo cell division or peripheral deletion in ALB1 mice. It is important to stress that TCR-tg cells isolated from TCR × ALB1 double-tg or TCR × ALB1 × 107.5 triple-tg mice were able to proliferate and to induce disease after in vivo stimulation with Ag. In this respect, our experiments show parallels to models using tg mice expressing OVA (35) or LCMV glycoprotein/nucleoprotein (13, 14, 36) in the β-cells of the endocrine pancreas.

The mechanism of CD8 T cell tolerance to Ags in the liver has been previously addressed by Bertolino et al., who reported activation followed by peripheral deletion of K b-specific TCR-tg cells in irradiation chimeras generated with tg mice expressing K b in the liver and lymph node cells from TCR-tg mice (22). In contrast, Schönrich et al. observed tolerance induction by down-regulation of the TCR on self-reactive CD8 T cells in tg mice expressing K b in the liver under control of the albumin promoter (19). Hepatitis could be induced in this model when TCR-tg cells were activated by strong antigenic stimuli (Ag plus IL-2) and when inflammatory signals induced by infectious agents (Listeria monocytogenes) were provided (37). Ferber et al. further demonstrated that the extent of down-regulation of the tg K b-reactive TCR correlated with the amount of K b expression on liver cells (20).

How can the differing results in the K b and LCMV GP33 models be explained? In the system described here, a MHC class I-restricted peptide Ag (GP33) was employed as a model Ag, whereas in the studies referred to above, an allo-MHC class I Ag (K b) was used. Allo-reactive T cells recognize foreign MHC class I molecules in both peptide-dependent and -independent manners (38). It is therefore difficult to compare the number of target molecules on the cell surface of hepatocytes in the two tg models. In addition, the Ag affinities of the tg TCRs used may differ. Despite these limitations, we favor the explanation that expression of a neo-self Ag at high levels in the liver leads to active tolerance mechanisms (i.e., TCR down-regulation, anergy, peripheral deletion), whereas expression at a lower level, as shown here, leads to ignorance. A similar conclusion has recently been reached from studies using tg mice expressing OVA under the control of the rat insulin promoter in the endocrine pancreas (35). However, the liver differs from the pancreas not only in size, but also in its architecture. The microvasculature of the liver is distinct from that seen in most other tissues, due to the discontinuous endothelial cell layer and the lack of a basal membrane, which may render hepatocytes particularly susceptible to recognition by CD8 T cells (15).

It is well established that the migration of T cells through lymphoid and nonlymphoid organs is crucially influenced by their activation state (39). We observed a dramatically increased infiltration of activated TCR-tg into the liver of ALB1 mice compared with naive TCR-tg cells (Fig. 6). This result is very much in line with several previous studies reporting accumulation and selective retention of activated T cells by the liver (40, 41). The poor infiltration of naive TCR-tg cells into the liver tissue of ALB1 mice may help to explain how tolerance by ignorance is established in this tg model. After LCMV infection, TCR-tg cells are activated and gain the ability to efficiently infiltrate the liver to cause disease. The question, however, of what proportion of TCR-tg cells present in adult TCR × ALB1 double-tg mice has been in contact with GP33-expressing hepatocytes once in their lifespan remains open.

Infection of normal mice with a high dose (105 PFU) of LCMV induces CD8 T cell-mediated acute hepatitis (42). It must be emphasized that the tg model described here is distinct from this latter experimental system. In our model, recipient mice of TCR-tg cells are infected with a low dose of LCMV (200 PFU), and this infection induces hepatitis only in ALB1 or ALB1 × 107.5 tg mice, but not in normal or 107.5 single-tg mice. Thus, the increased transaminase levels in the mice examined here are due to an autoimmune response directed against the tg neo-self Ag. In the former system the increased transaminase levels at high dose infection are due to a CTL response against LCMV-infected hepatocytes.

T cells play a crucial role in the pathogenesis of virus-induced liver diseases. However, only a few murine models of virus-induced autoimmune hepatitis have been described to date. In the HBsAg tg model, adoptively transferred activated T cells from HBsAg-treated donor mice cause transient hepatitis in tg mice expressing HBV envelope proteins in hepatocytes (43–45). In contrast to the model described here, active immunization of these mice fails to induce autoimmune hepatitis (18, 46) due to tolerance induction by as yet undefined mechanisms.

Hepatocytes in 107.5 tg mice store the tg HBsAg in the endoplasmic reticulum, which has been shown to render these cells sensitive to cell death induced by several inflammatory stimuli (LPS, IFN-γ, or TNF-α) (30). The question, however, of to what extent the increased sensitivity of HBsAg tg hepatocytes contributes to the T cell-mediated hepatitis could not yet be directly addressed. We were able to dissect this process by using either ALB1 (specific Ag), 107.5 (sensitive hepatocytes), or ALB1 × 107.5 (specific Ag and sensitive hepatocytes) double-tg mice as recipients for TCR-tg cells. Importantly, 107.5 single-tg mice did not exhibit significantly increased transaminase levels (&lt;50 U/L), indicating that the inflammatory cytokines (IFN-γ and TNF-α) produced during LCMV infection were not sufficient to induce hepatitis by bystander effects. However, serum transaminase levels in LCMV-infected ALB1 × 107.5 double-tg recipient mice (600–700 U/L) were considerably higher than those in ALB1 single-tg mice (200–300 U/L), indicating that HBsAg storage disease (“ground glass” hepatocytes) plays an important role in the pathophysiology of HBV infection.

In conclusion, we describe here a new model of CD8 T cell-mediated autoimmune hepatitis induced by a viral infection. This tg mouse model may serve as a valuable tool to examine the immunopathologic processes of T cell-mediated hepatitis.

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References


