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Hepatitis resulting from liver-specific expression and recognition of self-antigen

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Abstract

Liver-specific immune reactivity in response to aberrant expression of antigen on the surface of hepatocytes is thought to be a major factor in development of autoimmune hepatitis (AIH). Persistent inflammation develops when these antigens are not eliminated and/or responses are not appropriately regulated. We have developed transgenic mice (OVA-HEP), which express chicken ovalbumin on the surface of hepatocytes. These mice are tolerant to ovalbumin, develop normally and have shown no evidence of liver or other disease up to 2 years of age. Adoptive transfer of naïve ovalbumin specific T cells into OVA-HEP transgenic mice led to liver specific inflammation in a dose dependent manner. This hepatic necroinflammation was dependent upon CD8+ V α 2 OVA specific T cells; was limited to the liver; and was augmented by OVA-specific CD4+ T cell help; but did not result from adoptive transfer of ovalbumin specific CD4 T cells alone. The response was self limited but persistent inflammation developed after repeated transfer of antigen specific T cells. This model of T cell recognition of antigen on hepatocytes may be used to understand many liver-specific aspects of the immune response in autoimmune hepatitis.

Keywords

antigen-specific; autoimmunity; Autoimmune Hepatitis (AIH); acute hepatitis; chronic hepatitis

1. Introduction

An important mechanism for generation of liver-specific immune responses is aberrant expression of normal or foreign antigens on the surface of hepatocytes. While normally, self-antigens do not induce an immune response, this is not true when tolerance is lost or antigens that are not exposed to the immune system become so exposed. In humans the resulting immune response is postulated to include both antigen specific T cells and non-specific liver immune responses and ultimately may lead to chronic disease. We have developed transgenic mice (OVA-HEP), which express a well-characterized protein, ovalbumin (OVA) on the surface of hepatocytes. These OVA-HEP mice are tolerant to OVA because of antigen expression during T cell development. OVA-HEP mice develop normally and have shown no evidence of liver

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or other disease up to two years of age. However, adoptive transfer of OVA specific CD4⁺ and CD8⁺ T cells results in liver inflammation which continues for over 4 months. CD8 cells are required for the development of acute and chronic hepatitis and OVA-specific CD4 T cells enhance the hepatitis but do not induce hepatitis alone.

2. Materials and Methods

2.1 Construction of the plasmid

Alb/OVA cDNA was constructed using albumin (Alb) promoter [1], a membrane-bound ovalbumin (aminoacid 139-385 [2]) and a polyA tail (pDo15-polyA). The membrane bound form of OVA consists of a fusion protein made up of the first 118 residues of the human transferrin receptor (including cytoplasmic tail and signal/anchor domain) linked to residues 139-385 of mature OVA (TFR-OVA), targeting membrane expression of OVA on hepatocytes. The TFR-OVA fragment was excised from the RIP-mOVA plasmid by digestion with HindIII and XbaI (all restriction enzymes from New England Biolabs, Ipswich, MA). The Alb promoter and TFR-OVA fragments were then ligated into the pBluescript vector (Stratagene, La Jolla, CA). The polyA region was a rabbit β -globin gene isolated from the pDOI-5 plasmid from BamHI to XhoI and was blunted at two ends[3] and inserted into the pBSSK-Alb-mOVA. Its orientation was checked after insertion. After isolation of the plasmid, restriction enzyme mapping was performed and TFR-OVA was sequenced to verify that the construct was correct. Vector sequences were excised by the digestion with NotI and XhoI. The purified DNA was microinjected into pronuclei of the C57/BL6 oocytes (Jackson laboratory, Bar Harbor, Me).

2.2 Development of transgenic mice

C57/BL6 (H-2^b) oocytes were injected with the Alb/OVA DNA. Weanling mouse tail DNA of 54 mice was extracted and were tested by PCR for membrane bound ovalbumin (OVA^m). Primers for Alb-OVA were 5' CCTTCAGCCAAGCTCCGTGGATTCT and 3' CCAGACAGATTGGCTGAAGAGCTA. 30 cycles (denaturation (95 °C 45 sec), primer annealing (55°C for 45 sec) and primer extension (72 °C for 1 min) produced a single band of 460 bp on ethidium bromide stained 1% agarose gels. Tail DNA from OVA-HEP mice was positive for the transgene by PCR, and was confirmed by Southern Blot analyses: 6 founder lines of OVA-HEP were chosen. In some experiments OT-I and OT-II mice were crossed to RAG-1^{-/-} background to prevent endogenous rearrangement of T cell receptors and thus avoid generation of CD4⁺ and CD8⁺ T and B lymphocytes. For some experiments, OVA-HEP mice were crossed to B6.PL-Thy1^a/Cy mice (Jackson Laboratories), thereby acquiring Thy1.1 molecules. Mice were housed in sterile isolator cages maintained under clean, specific pathogen-free conditions, according to NIH guidelines (Publication 86-23). Animal Studies Committees of University of California, San Francisco and Washington University School of Medicine approved the protocol.

2.3 Adoptive cell transfer system

OT-I T cell receptor transgenic mice (Jackson laboratory, Bar Harbor, ME) produce OVA specific, MHC Class I H-2^b restricted CD8⁺ cytolytic T cells (OT-I T cells) and recognize the SIINFEKL peptide sequence at residues 257-264 of OVA. OT-II T cell receptor transgenic mice produce MHC Class II I-A^b restricted CD4⁺ helper T cells (William Heath and J.FAP Miller, the Walter and Eliza Hall Institute, Melbourne, Australia) and recognize a different T cell epitope, OVA (323-339 /I-A^b). OT-I and OT-II transgenic cells utilize V α 2 V β 5 as their T cell receptor (TCR) compared with <2% of non-transgenic C57/BL6 mice. Since there is no clonotypic antibody, mAb staining for V α 2 was used to identify adoptively transferred OVA specific T cells in OVA-HEP mice. OT-I and OT-II T spleen cells are homozygous for Thy1.2. Thus after adoptive transfer of OT-I or OT-II spleen cells into OVA-HEP/Thy1.1^{+/+} mice, double staining for Thy1.2 and CD8 or CD4 was also used to identify T cells of donor (OT-I

or OT-II) origin. Varying numbers of T cells from OT-I and OT-II mice or normal controls were injected intravenously (iv) or intraperitoneally (ip) into OVA-HEP transgenic mice or control C57/BL6 mice. Serial serum alanine aminotransferase levels (ALT) were performed using the GP Transaminase kit (Sigma, St Louis, MO) to evaluate liver inflammation. At various time points, mice were sacrificed and histologic sections of the liver were studied for evidence of portal and lobular inflammation [4].

2.4 Immunohistochemical analyses

Mice were sacrificed at varying days after T cell transfer and tissues were placed in neutralized 10% formalin solution (J.T.Baker, Phillipsburg, NJ) or frozen in Optimal Cutting Temperature compound (OCT; Miles Inc, IN). Briefly, cryostat sections of frozen tissues were fixed in ethanol at 4°C for 10 minutes, air dried and stored at 20°C. Rabbit anti-OVA antibody was obtained by immunizing rabbits with whole OVA (OVA grade V, Sigma, St. Louis, MO) and was used to confirm OVA expression on hepatocytes. Sections were pre-incubated in blocking serum for 15 minutes and then incubated with rabbit anti-OVA antibody (40 µg/ml) at 4°C overnight followed by incubating with FITC or PE conjugated anti-rabbit IgG. Double staining was carried out by staining the tissue sections with purified mAb to CD4 (RM4-5) or CD8 (53-6.7) (BD Pharmingen, San Diego, CA) followed by Cy3 conjugated goat anti-rat IgG (Jackson ImmunoResearch, West Grove, PA). Tissues sections were then incubated with FITC conjugated rat anti-mouse Thy1.2 antibody (53-2.1; BD Pharmingen, San Diego, CA).

2.5 Hepatic Mononuclear Cells Isolation and FACS analyses

Livers were perfused with collagenase (0.1 mg/mL in PBS; Sigma, St Louis, MO) via the portal vein and mononuclear cells (MNC) isolated as described [5] on a discontinuous percoll gradient. The interface contained mononuclear >95% CD45 positive by flow cytometry. Spleens were excised and homogenized through a 70 micron filter. Liver and spleen MNC cells were counted, centrifuged and raised in FACS buffer (15.4 µM NaN₃ in 2% FBS/PBS) to a concentration of $5-15 \times 10^6$ cells/cc and treated with anti-mouse CD16/CD32 (1 µg/million cells) to inhibit Fc mediated binding (all monoclonal antibodies (mAb) from BD Pharmingen, San Diego, CA). MNC were labeled for 45 minutes with fluorescein (FITC), phycoerythrin (PE), PE-Cy5.5, and APC conjugated mAb to MNC (CD45), T lymphocytes (CD3, CD4, and CD8), TCR Vα2, Thy 1.1 and Thy 1.2. They were subsequently washed with FACS buffer and analyzed by flow cytometry as described[5].

Cell division in vivo was measured by labeling OT-I and OT-II splenocytes with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR) as described[5]. Briefly, OT-I or OT-II spleen cells at a concentration of 2×10^7 cells/cc were labeled with CFSE in DMSO (1 mg/cc) for 5 minutes at 37° C. The reaction was quenched with 10% FBS/RPMI, washed, confirmed to be > 99% CFSE labeled by flow cytometry, and injected into OVA-HEP mice. At varying times, the mice were sacrificed and the liver and spleen MNC analyzed by flow cytometry using phycoerythrin conjugated mAb to CD4 and CD8. CFSE labeled OT-I or OT-II mice were injected alone and with unlabeled OT-II or OT-I cells.

3. Results

3.1 Development of Transgenic OVA-HEP mice

C57/BL6 (H-2^b) oocytes were injected with pBSSK-Alb/OVA cDNA. Mouse tail DNA of 54 mice was extracted and tested by PCR for membrane bound ovalbumin (mOVA). 24 of the 26 PCR positive tail DNA were confirmed by Southern Blot analyses: 6 founders lines (OVA-HEP) were chosen. One medium and one high expressing founder line were expanded for adoptive transfer experiments. Tissues from heart, liver, kidney, colon, lung and brain were

tested for expression of OVA by immunofluorescence. OVA mAb only stained the hepatocytes of these mice (Fig. 1). Although the thymus did not stain for OVA by immunohistochemistry, RT-PCR analysis revealed low level OVA mRNA expression (data not shown). There was no liver inflammation as assessed by serum ALT and histology of OVA-HEP transgenic mice housed up to two years of age.

3.2 Adoptive transfer of naïve OVA specific T cells into OVA-HEP mice led to necroinflammation of the liver

Lobular and portal inflammation resulted from adoptive transfer of naïve T cells from CD8⁺OT-I and CD4⁺OT-II transgenic mice into OVA-HEP mice. 5×10^6 OT-I T cells and 2×10^6 OT-II T cells injected ip into OVA-HEP mice led to elevation of serum ALT that peaked at day 3 and returned towards normal range by day 14 (Fig. 2A) and baseline by day 21 (data not shown). Figure 3 depicts histology of OVA-HEP livers at day 3, 5, and 7 after adoptive transfer of OT-I and OT-II T cells. This inflammation was both portal and lobular with mixed mononuclear cell infiltration, apoptotic hepatocytes, interface hepatitis and ballooning degeneration. Tissues from heart, kidney, colon, lung, brain and pancreas did not reveal any inflammatory infiltrate (data not shown). Similar elevations of ALT and necroinflammation on immunohistochemistry were noted in OVA-HEP mice after adoptive transfer of OT-I and OT-II splenocytes from wild type or RAG deficient OT-I and OT-II mice (data not shown). Adoptive transfer of similar numbers of OT-II T cells alone (without OT-I splenocytes) did not lead to liver inflammation as assessed by ALT (Fig. 2B) or histology (Table 1). Similar transfer of OT-I and OT-II T cells into C57/BL6 normal mice did not produce liver inflammation nor was there any elevation in serum ALT (Figs. 2A, 3F, Table 1). In addition, transfer of normal C57/BL6 T cells (primed or unprimed with OVA) into OVA-HEP mice resulted in no inflammation (Table 1). Thus the hepatitis seen was restricted to CD8⁺ OVA-specific T cells adoptively transferred into OVA expressing recipient mice. There was no difference in the level of liver inflammation and serum ALT whether adoptive transfer was performed i.p or i.v. In 16 OVA-HEP mice receiving adoptively transferred T cells i.v., peak ALT was 124 ± 45 IU/l and in 37 OVA-HEP mice receiving adoptively transferred T cells i.p., peak ALT was 100 ± 27 IU/l. Similar levels of liver inflammation were obtained from different OVA-HEP transgenic founder lines after adoptive transfer of OT-I and OT-II T cells and one OVA-HEP line was expanded for further studies.

3.3 Chronic inflammation develops after repeated adoptive transfer of OT-I and OT-II T cells into OVA HEP mice

A single injection of OT-I and OT-II T cells led to elevated serum ALT and liver inflammation for 14-21 days. Repeated transfer of OT-I and OT-II T cells (every 2 weeks for 3 injections in total) resulted in a chronic hepatitis for at least 12 weeks duration. During the first six weeks, serum ALT levels were consistently elevated in all mice. After this time ALT levels declined with 40% of mice having elevated serum ALT at 9 weeks, and 30% at 12 weeks. Histology revealed foci of infiltration by mononuclear cells that decreased in intensity with time from T cell transfer (Fig. 4) and varied between mice. However, all of the mice had at minimum focal areas of lobular and portal inflammation at 10-12 weeks, even those with near normal or normal range serum ALT levels.

3.4 OVA-specific T cells home to OVA-HEP liver and proliferate

To assess homing and proliferation, OT-I splenocytes or purified CD8 T cells were labeled with CFSE prior to adoptive transfer either alone or with unlabeled OT-II splenocytes. At 3 days the CFSE-labeled OT-I splenocytes were found to be vigorously dividing in the liver but not in the spleen (Fig. 5). At least 4 discrete divisions occurred during this time period. OT-II

splenocytes labeled with CFSE and adoptively transferred without OT-II cells did not proliferate (data not shown).

3.5 OVA-specific CD8⁺ lymphocytes expand in OVA-HEP liver after single or multiple adoptive transfer of OT-I and OT-II splenocytes

After adoptive transfer of OT-I and OT-II spleen cells into OVA-HEP mice, the liver and spleen MNC were isolated and assessed by flow cytometry. Consistent with histologic and biochemical data, the number of OVA specific T cells (V α 2 CD8⁺ lymphocytes) increased markedly following adoptive transfer of OT-I and OT-II but returned to baseline within 3 weeks (Figure 6A). In contrast, mice injected repeatedly with OVA-specific T cells at weeks 0, 2, and 4 had a persistently elevated V α 2 CD8 T cell levels. There were $0.7 \pm 0.1 \times 10^6$ V α 2 CD8 T cell in OVA-HEP mice at day zero (prior to adoptive transfer); $3.8 \pm 0.7 \times 10^6$ V α 2 CD8 T cells at 12 weeks and $2.7 \pm 0.8 \times 10^6$ V α 2 CD8 T cells 16 weeks after repeated adoptive transfer of OVA-specific T cells (Fig. 6B). These findings are consistent with the inflammation noted on histology and seen up to 120 days in mice receiving multiple adoptive transfers of OVA-specific T cells.

To further confirm the origin and specificity of the inflammatory infiltrate, we crossed OVA-HEP transgenic mice with Thy 1.1 C57/BL6 mice, so that all T cells are positive for Thy1.1. Adoptive transfer of OT-I/Thy 1.2⁺ and OT-II/Thy 1.2⁺ T cells into OVA-HEP/Thy1.1⁺ mice resulted in similar elevation of ALT and hepatitis as that seen with OVA-HEP/Thy1.2⁺ mice (data not shown). Peak serum ALT occurred at day 3 after adoptive transfer of OT-I and OT-II T cells.

Double staining of adjacent sections of OVA-HEP liver with CD4 mAb or CD8 mAb and with Thy1.2 antibody was performed (Fig. 7). This revealed the presence of both CD4⁺ and CD8⁺ T cells. The majority of the CD8⁺ T cells stained double positive for Thy1.2 and CD8 and thus were derived from OT-I mice (Figs. 7A-C). Few CD4⁺ T cells stained double positive for CD4 and Thy1.2. The liver and spleen MNC populations were also assessed by flow cytometry following adoptive transfer. Consistent with immunohistochemical analyses, the population of double positive Thy 1.2 and CD8 T cells expanded in the liver but not the spleen (Fig. 7D), while the population of CD8 positive Thy 1.2 negative cells, representing the host CD8 T cell population, did not increase. Taken together, these data show expansion of adoptively transferred OVA specific CD8 T lymphocytes in the liver of OVA expressing mice.

4. Discussion

These studies show that T cell recognition of antigen expressed on hepatocytes leads to liver specific inflammation, which is both acute and chronic. This inflammation is predominantly periportal with mononuclear cell infiltration, interface hepatitis and liver cell apoptosis. Acutely, there are numerous OVA specific CD8 T cells infiltrating the liver with liver cell necrosis as measured by high serum ALT and necroinflammation in the liver. Repeated injections of OVA specific T cells established a chronic mononuclear cell inflammation with ongoing hepatocyte damage: inflammation was both portal and lobular. These studies show that antigen specific T cell recognition of aberrant antigen can initiate and perpetuate liver disease. Our model differs from previously published work because in addition to studying induction of acute disease, we will be able to study the role of antigen specific T cells in perpetuation of chronic disease.

Auto recognition of self-antigen is thought to be the hallmark of autoimmune liver diseases. Many autoantigens have been implicated in AIH including various cytochrome P450 antigens, actin, chromatin and glucuronyl transferase[6]. However, the resulting liver pathology is similar, showing chronic active hepatitis with mononuclear cells infiltration, piecemeal

necrosis and liver cell rosetting. The precipitating events, as well as the regulation of autoimmune disease in the liver, remains unresolved although there is evidence for HLA association with DR3 and DR4 [7]. T cell cytotoxicity and up regulation of Class I on hepatocytes have been shown [8;9] with activated CD4 and CD8 T cells in periportal areas. Genetic predisposition appears to be required but is not sufficient to induce disease, suggesting a “second hit” is needed. In general, studies of pathogenesis of AIH are hampered by the lateness of presentation of patients, many years after the initiation of disease.

There are a number of models of autoimmune and viral induced liver diseases which have been developed in rodents and rabbits in response to liver antigens [10;11]. Early experiments of C57/BL6 mice immunized with syngeneic liver antigen developed hepatitis, which was T cell mediated and self limited [12-14]. Autoimmune polyglandular syndrome type 1 has been shown to be associated with mutations in the AIRE gene[15], but only few patients with autoimmune hepatitis, without the polyglandular syndrome, have been shown to have mutations in this gene[16]. In addition, in AIRE^{-/-} mice, there is great variation in organ specific expression of disease, depending upon the genetic background of the mice studied[17].

Transgenic and knock out models have allowed for major advances in studies of both autoimmunity and tissue specific immunity. Unfortunately the transgene is seen as self in transgenic models so that the immune environment is tolerized to the transgene during development. T lymphocytes, which recognize antigens on the cell surface, are deleted or develop anergy. Enough antigen is expressed, even if undetectable by routinely employed biochemical and immunohistochemical methods, such that the mice become tolerant to the transgene product. There is immune tolerance in our OVA-HEP-expressing mice. To overcome tolerance, transgenic mice expressing T cell receptor genes from OVA specific T cells were developed by Carbone and colleagues: OT-I T cells are CD8+, MHC Kb Class I restricted and OT-II T cells are CD4+ MHC I-Ab Class II restricted [3]. OT-I and OT-II recognize distinct but overlapping OVA peptides. Adoptively transfer of these naïve antigen-specific T cells, induced liver targeted inflammation. These reagents have been utilized to study biliary directed inflammation in transgenic mice with OVA expressed on biliary epithelial cells[5].

Elegant transgenic models of Hepatitis B and C have been developed by Chisari et al [18]. Transgenic mice express viral antigens in the liver. These mice are tolerant to HBsAg but develop liver inflammation after adoptive transfer of syngeneic specific cytolytic T cells and cytokines [19;20]. Our transgenic model differs in that there is no viral replication and no intracellular antigen as OVA is membrane bound. Voehringer et al developed another model in which the CTL epitope GP33 of lymphocytic choriomeningitis virus (LCMV) was expressed in the liver under control of the albumin promoter [21]. As was noted with our OVA-HEP mice, high antigen liver expression and low thymic expression was found in these mice. But there was incomplete deletion of GP33 specific T cells from their mice with evidence of peripheral ignorance. Addition of cytolytic T cells specific for GP33 to their transgenic mice did not result in liver inflammation. CTLs had to be activated by infection with LCMV prior to adoptive transfer and then hepatitis developed in the GP33 transgenic mice. Thus expression of their antigen within the liver did not lead to activation of specific CTL perhaps because there was no surface expression of GP33, so that viral cytolysis was required to initiate inflammation in this model. Our model differs in that surface expression of OVA allows for recognition by OT-I T cells.

Bertolino et al used Met-K^b transgenic mice which express H-2 K^b gene in hepatocytes under control of the sheep metallothionein promoter to study activation of CD8+ T cells within the liver [22]. Adoptively transferred H-2 K^b specific CD8+T cells homed to the liver of these transgenic mice within 2 hours of transfer, underwent cell division and recirculated within 24 hours. Liver inflammation was brief, occurring only in the first 24 hours and the life span of

the T cells was short lived [23]. These findings differ from Crispe et al who found liver inflammation in the absence of hepatocyte antigen presentation, suggesting that the liver is a “sink” for activated CD8+ T cells and that cytotoxicity is due to a bystander effect [24;25]. These investigators have only studied acute events and required in vitro activated T cells. In our model, naïve OVA-specific T cells do not require activation prior to adoptive transfer of OT-I T cells in order to induce hepatitis and inflammation was not induced when activated C57/BL6 splenocytes were transferred into OVA-HEP mice.

In conclusion, our model is similar to other transgenic models in that there is immune tolerance in transgene-expressing mice and thus no liver disease without adoptive transfer of naive antigen specific T cells. Our model differs from previously published work because we have antigen expressed on the surface of hepatocytes and do not require activation of T cells in vitro to induce liver specific inflammation. Thus we are able to study the role of antigen specific T cells in induction of acute disease and perpetuation of disease. This model can be used to expand our knowledge of liver specific immunity and its dysregulation, which is of great importance in many diseases of the liver, including autoimmune hepatitis.

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Figure 1. Expression of OVA on hepatocytes of OVA-HEP transgenic mice. Immunofluorescent staining of liver using rabbit anti-OVA Ab showed uniform staining of hepatocytes. The original magnification is 200 $\times$.

Figure 2.

Serum ALT increases following adoptive transfer of OVA-specific spleen cells into OVA-HEP mice. (A) serum ALT (IU/L) increases at varying days after adoptive transfer of 5×10^6 OT-I and 2×10^6 OT-II T cells into OVA-HEP mice (closed diamonds) or normal C57/BL6 (open squares). Mean $\pm$ SEM of 3 mice per time point. (B) serum ALT (IU/L) does not change at varying days following the adoptive transfer of 2×10^6 OT-II (closed diamonds), 5×10^6 OT-II (open squares), or 10×10^6 OT-II (open triangles) into OVA HEP mice. Mean $\pm$ SEM of 3 mice per time point.

Figure 3.

Liver histology of OVA-HEP mice at varying times after single adoptive transfer of OT-I and OT-II T cells. Portal and lobular mononuclear cell infiltration were observed at day 3 (A and B, 400 $\times$) and (C, 200 $\times$), day 5 (D, 200 $\times$), and day 14 (E, 200 $\times$) in OVA-HEP mice adoptively transferred with OT-I and OT-II T cells. (A) and (B) show two different portal tracts with mononuclear cell infiltration, swollen and apoptotic hepatocytes, interface hepatitis and lobular infiltration (C). (E) shows lobular mononuclear cells infiltrating and surrounding apoptotic hepatocytes with central veins approximation (D). (F) shows normal liver histology at day 7 after transfer the OT-I and OT-II T cells to normal C57/BL6 mice (100 $\times$).

Figure 4. Liver histology of OVA-HEP mice from 8-12 weeks after repeated adoptive transfer of OT-I and OT-II T cells on days 0, 14, and 28. Both portal and lobular inflammation is seen 8 weeks (A and B) and 12 weeks (C and D) after adoptive transfer of OT-I and OT-II T cells into OVA-HEP mice. (A)-(D) show mixed mononuclear cell infiltration around the portal tracts and lobules. (C) shows a more severe mononuclear infiltrate at week 12 in a mouse with a consistent elevated serum ALT. Magnification (A, 100 $\times$), (B, 200 $\times$), (C and D, 100 $\times$).

Figure 5.

Proliferation of CFSE-labeled OT-I splenocytes 3 days after adoptive transfer with unlabeled OT-II splenocytes into OVA-HEP mice. CD8 T cells in the liver (red) are shown to have undergone a multiple discrete divisions while few CFSE stained OT-I are noted in the spleen (blue) without evidence of cell division. Representative plot of 4 experiments.

Figure 6.

Expansion of CD8 Vα2 T cells following single and multiple adoptive transfers of OVA specific T cells into OVA-HEP mice. (A) CD8 Vα2 T cells increased markedly over 7 days in OVA-HEP liver, following a single adoptive transfer of 5×10^6 OT-I and 2×10^6 OT-II splenocytes as measured by flow cytometry. CD8 Vα2 T cells return to baseline by day 21. Mean $\pm$ SEM of 22 total mice. (B) After repeated adoptive transfer of 5×10^6 OT-I and 2×10^6 OT-II splenocytes into OVA-HEP mice at days 0, 14, and 28, CD8 Vα2 liver T cells expand and remain significantly elevated above baseline for 16 weeks. Mean $\pm$ SEM of 17 total mice.

Figure 7.

Expansion of CD8 Thy 1.2 T cells following adoptive transfer of OVA-specific T cells into OVA-HEP mice. (A) and (B) show immunofluorescent staining of adjacent liver sections from OVA-HEP mice with FITC-labeled anti-Thy 1.2 mAb (A, 200 $\times$) and purified anti-CD8 mAb followed by Cy3 conjugated anti-Rat Ab (B, 20 $\times$) 3 days after injection of 5×10^6 OT-I/RAG and 2×10^6 OT-II/RAG T cells into OVA-HEP Thy1.1^{+/+} mice. (C) is an overlay of these two stains showing the majority of infiltrating cells are double stained with Thy1.2 and CD8. (D) shows the expansion of CD8 and Thy 1.2 positive cells in the liver (red line) but not the spleen (blue line) at days 3, 5, and 7 following adoptive transfer of 5×10^6 OT-I RAG and 2×10^6 OT-II RAG T cells into the OVA-HEP Thy 1.1^{+/+} mice as measured by flow cytometry. Representative of 4 mice per time point.

Table 1
Liver inflammation after adoptive transfer of OVA specific CD4+ (OT-II) and CD8+ (OT-I) T cells into recipient mice

T cells transferred	Recipient mice	Serum ALT		N	H&E Liver
<i>Experiment I</i>		Day 0	Peak ALT		
0.5 × 10 ⁶ OT-I 2 × 10 ⁶ OT-II	OVA-HEP	16 ± 3	41 ± 12	3	Inflammation
5 × 10 ⁶ OT-I 2 × 10 ⁶ OT-II	OVA-HEP	14 ± 6	131 ± 44	3	Inflammation
2 × 10 ⁶ OT-II	OVA-HEP	17	18	1	None
<i>Experiment II</i>					
2 × 10 ⁶ OT-II	OVA-HEP	9 ± 2	13 ± 1	2	None
5 × 10 ⁶ OT-II	OVA-HEP	12 ± 2	12 ± 1	2	None
10 × 10 ⁶ OT-II	OVA-HEP	8 ± 2	18 ± 7	2	None
5 × 10 ⁶ OT-I 2 × 10 ⁶ OT-II	OVA-HEP	8 ± 3	74 ± 26	7	Inflammation
<i>Experiment III</i>					
5 × 10 ⁶ C57/BL6	OVA-HEP	8 ± 1	8 ± 2	3	None
5 × 10 ⁶ C57/BL6 (OVA primed)	OVA-HEP	6 ± 1	10 ± 1	3	None
5 × 10 ⁶ OT-I 2 × 10 ⁶ OT-II	C57/BL6	7 ± 7	13 ± 3	3	None
5 × 10 ⁶ OT-I 2 × 10 ⁶ OT-II	OVA-HEP	10 ± 3	90 ± 29	8	Inflammation