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IL-22-Dependent Attenuation of T Cell-Dependent (ConA) Hepatitis in Herpes Virus Entry Mediator Deficiency

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Coinhibitors and costimulators control intrahepatic T cell responses that trigger acute hepatitis. We used the ConA-induced hepatitis model in the mouse to test if the coinhibitor herpes virus entry mediator (HVEM) modulates hepatitis-inducing T cell responses. Compared with ConA-injected, wild-type (wt) C57BL/6 (B6) mice, HVEM-deficient (HVEM−/−) B6 mice showed lower serum transaminase levels and lower proinflammatory IFN-γ, but higher protective IL-22 serum levels and an attenuated liver histopathology. The liver type I invariant NKT cell population that initiates acute hepatitis in this model was reduced in HVEM−/− mice but their surface phenotype was similar to that of untreated or ConA-treated wt controls. In response to nitrogen injection, liver invariant NKT cells from HVEM−/− B6 mice produced in vivo more IL-22 but lower amounts of IFN-γ and IL-4 than wt controls. Bone marrow chimeras showed that HVEM deficiency of the liver nonparenchymal cell population, but not of the parenchymal cell population, mediated the attenuated course of the dendritic cell- and T cell-dependent ConA hepatitis. IL-22 is produced more efficiently by liver NKT cells from HVEM−/− than from wt mice, and its Ab-mediated neutralization of IL-22 aggravated the course of hepatitis in wt and HVEM−/− mice. Hence, HVEM expression promotes pathogenic, proinflammatory Th1 responses but down-modulates protective IL-22 responses of T cells in this model of acute hepatitis. The Journal of Immunology, 2009, 182: 4521–4528.

Intravenous injection of the T cell mitogen ConA into mice induces an acute, T cell-dependent hepatitis (1). The induction of this hepatitis depends on CD1d-restricted invariant NKT (iNKT) cells (that express an invariant Vα14Jα18 TCR) and is supposed to be CD8 T cell-independent (2). A main feature of the disease is the TNF-α/TNFRI- and/or CD95/CD95L-mediated acute injury of hepatocytes (3–7). Cytokines are key mediators in the induction, course, and severity of this disease. Disease-aggravating cytokines are TNF-α and IFN-γ (3, 4), IL-4 (2, 7, 8), IL-5 (9), leptin (10), and LIGHT/LTβR signals (11). Disease-attenuating cytokines are IL-6 (3, 12), IL-10 (13, 14), IL-27 (15), and IL-22 (16). It is unclear which ConA-presenting cell stimulates through which costimulatory/coinhibitor pathway(s) the pathogenic T cell response in this model.  

Herpes virus entry mediator (HVEM), also designated TNFRSF14, binds the TNF superfamily homologue LIGHT (TNFSF14), the CD28 family homologue B and T lymphocyte attenuator (BTLA) (17), and CD160. HVEM is expressed by most cells of the immune system (T cells, B cells, NK cells, dendritic cells (DC), and monocytes) and by some nonimmune cells such as hepatocytes, intestinal epithelial cells, and smooth muscle cells. The widespread expression of the HVEM receptor and its ligands by many cell types at different stages of their activation/differentiation makes it difficult to elucidate their role in regulating T cell responses. Compared with wild-type (wt) T cells, HVEM−/− T cells show enhanced responses to ConA stimulation in vitro and in vivo (18). HVEM−/− mice show increased susceptibility to experimental autoimmune encephalomyelitis (18), an IL-17-producing (Th17) CD4 T cell-dependent immunopathology (19). Interaction of HVEM with its ligands may regulate the magnitude but also the polarization of pathogenic T cell responses.  

Th17 cells represent a recently identified T cell lineage distinct from the Th1 and Th2 effector CD4 T cell lineages (20). Costimulatory or coinhibitory signals that guide the differentiation of this lineage are largely unknown. Th17 cells produce substantial amounts of IL-22 (21) that attenuate immune injury inflicted on parenchymal cells in ConA hepatitis (16). We observed an attenuated course of ConA hepatitis in HVEM−/− as compared with wt mice. This is at least partly explained by the observation that HVEM deficiency within nonparenchymal cell (NPC) populations in the liver efficiently supports development of IL-22-producing cells.

Materials and Methods  

Mice  

C57BL/6 (B6) mice, HVEM−/− B6 mice (18), and transgenic CD11c.DTR B6 mice were bred and kept under standard pathogen-free conditions in the animal colony of Ulm University (Ulm, Germany). HVEM−/− B6 mice were provided by K. Pfeffer (Duesseldorf, Germany). Female and male mice were used at 8–10 wk of age. All animal experiments were approved by the local Institutional Animal Care and Use Committee in accordance with federal, provincial, and local regulations.
Isolation of mouse liver cell subsets
NPC populations from the liver were isolated as described (22). CD4<sup>+</sup> T cells and CD4<sup>-</sup> cells were isolated from liver NPC populations by MACS (catalog no. 130-049-01; Miltenyi Biotec).

Antibodies
Four-color flow cytometry analyses were performed using a FACSCalibur (BD Biosciences). Data were analyzed using the FCS Express V3 software (De Novo Software). PE-conjugated anti-CD160 mAb CNX46-3 (catalog no. 12-1601) and PE-conjugated anti-BTLA mAb 6F7 (catalog no. AF582) from R&D Systems were used to neutralize mouse IL-22. All other Abs were obtained from BD Biosciences.

Intracellular cytokine staining
Cells were cultured for 2 h in the presence of 2.5 μg/ml brefeldin A (catalog no. 15870; Sigma-Aldrich), harvested, washed, surface stained with APC-conjugated anti-CD4 mAb RM4-5 and biotinylated anti-CD3 mAb (catalog no. 16-1052; BD Biosciences), fixed (2% paraformaldehyde), resuspended in permeabilization buffer (Hanks’ balanced salt solution, 0.5% BSA, 0.5% Saponin, 0.05% sodium azide), incubated for 30 min with PE-conjugated anti-IL-22 mAb 140301, FITC-conjugated anti-IFN-γ mAb XGM1.2, PE-conjugated anti-IL-17 mAb TC11-18H10, or PE-conjugated anti-IL-4 mAb 11B11, washed twice, and resuspended in PBS (supplemented with 0.3% w/v BSA and 0.05% sodium azide).

CD1d/α-galactosyl-ceramide (αGalCer) dimers
Mouse NKT cells were labeled at 4°C for 15 min with divalent mouse CD1d-IgG1 fusion protein DimerX (cat. no. 557599; BD Biosciences) loaded with αGalCer (Alexis Biochemicals) and revealed with PE-coupled anti-mouse IgG1 (cat. no. 550083; BD Biosciences).

Determination of serum alanine aminotransferase (ALT)
Serum ALT levels were determined with a commercial kit (Roche Diagnostics) and are expressed as U/L.

Cytokine detection
Cytokines in serum or supernatants were detected by conventional double-sandwich ELISA using detection and capture Abs from BD Biosciences. IL-22 was detected by an immunoassay (cat. no. M2200; R&D Systems) and are expressed as U/L.

RT-PCR
Genomic DNA was removed with DNase from RNA prepared by the RNeasy mini Kit (Qiagen). RNA (2 μg) was reverse transcribed by SuperScript reverse transcriptase (Invitrogen) into cDNA that was submitted to real time PCR using the RT<sup>−</sup> SYBR Green supermix (SuperArray) and a standard quantitative PCR protocol. Quantitative real-time PCR was performed using the ABI PRISM 7500Fast sequence detector (Applied Biosystems). Hypoxanthine phosphoribosyltransferase or actin housekeeping genes were used to equalize cDNA amounts.

Bone marrow chimeras
Bone marrow cells from CD45.1 wt B6 or CD45.2 HVEM<sup>−/−</sup> donor mice were injected i.v. (4 × 10<sup>6</sup> cells/mouse) into lethally (9 Gy) irradiated CD45.1 wt B6 or CD45.2 HVEM<sup>−/−</sup> hosts. Chimeras were tested for reconstitution 8 wk posttransplantation with Abs to the appropriate allotypic marker (CD45.1 or CD45.2) and shown to contain >87% of spleen cells and liver NPC of donor type.

Statistical analyses
Data were analyzed using the GraphPAD prism software (version 4.0). Values are presented as means ± SD. The statistical significance of differences between groups was determined by the unpaired Student’s t test. Values of p < 0.05 and p < 0.005 are statistically significant.

Results
HVEM-deficient mice show an attenuated course of ConA hepatitis
Intravenous injection of ConA (20 mg/kg body weight) into 8–10-wk-old, wt B6 mice induced liver injury apparent as a rise in serum ALT and/or in serum cytokine (TNF, IFN-γ, and IL-22) levels in serum obtained 2, 4, 8, 16, 24, and/or 48 h postinjection were determined. Mean values (±SD) pooled from three independent experiments with three or four mice per time point per group. Corresponding groups marked with * (p < 0.05) or ** (p < 0.005) show a statistically significant difference. The difference between unmarked, corresponding groups is statistically not significant.

FIGURE 1. Attenuated ConA hepatitis in HVEM<sup>−/−</sup> mice. Wt and HVEM<sup>−/−</sup> B6 mice were i.v. injected with ConA (20 mg/kg). ALT (A) and cytokine (TNF, IFN-γ, and IL-22) (B) levels in serum obtained 2, 4, 8, 16, 24, and/or 48 h postinjection were determined. Mean values (±SD) pooled from three independent experiments with three or four mice per time point per group. Corresponding groups marked with * (p < 0.05) or ** (p < 0.005) show a statistically significant difference. The difference between unmarked, corresponding groups is statistically not significant.
transaminases (Fig. 1A) and severe liver histopathology, i.e., extensive areas of confluent hepatocellular necrosis, marked sinusoidal hyperemia associated with hemorrhage, and mononuclear cell infiltrates scattered diffusely throughout the viable parenchyma (data not shown). All wt mice injected with this dose of ConA survived. HVEM\(^{-/-}\) mice injected with the same dose of ConA survived and showed attenuated liver injury, i.e., lower rise in serum ALT levels (Fig. 1A) and less severe liver histopathology with only minor inflammatory infiltrates and small necrotic patches (data not shown). Serum cytokine levels in ConA-treated HVEM\(^{-/-}\) mice correlated with the attenuated course of this experimental hepatitis. Serum levels of the pathogenic cytokines

**FIGURE 2.** Liver iNKT cells. A, iNKT cells in liver, peripheral blood, and spleen from age/sex-matched wt and HVEM\(^{-/-}\) mice. The percentage of dimerX-binding iNKT cells in the gated CD3\(^+\) CD4\(^+\) T cell population from the indicated compartment of an individual, representative mouse per group. Three individual mice per group were analyzed. B, Surface phenotype of dimerX-binding liver iNKT cells from nontreated or ConA-treated, wt and HVEM\(^{-/-}\) mice. Liver NPC isolated 16 h postinjection were surface stained. Expression of the indicated marker by dimerX-binding liver iNKT cells from a representative mouse per group (of three individual mice per group analyzed) is shown. Dotted lines, isotype control; black lines, PBS-treated mice; gray filled curves, ConA-treated mice.
IFN-γ was lower whereas serum levels of protective IL-22 were higher in mitogen-injected HVEM−/− than wt mice (Fig. 1B). We detected no sex-related difference in the hepatitis response of wt and HVEM−/− B6 mice to ConA injection. ConA hepatitis in HVEM−/− mice was thus accompanied by lower systemic levels of the proinflammatory cytokine IFN-γ but higher levels of protective IL-22.

### Reduced numbers of iNKT cells with a different inducible cytokine profile are found in the liver of HVEM−/− mice

Because the induction of ConA hepatitis critically depends on iNKT cells, we compared the liver iNKT cell numbers in wt and HVEM−/− mice. The fraction of CD1dαGalCer dimer-binding CD4 iNKT cells in the liver CD4 T cell populations (Fig. 2A), as well as the absolute number of iNKT cells per liver (Table I), were reduced in HVEM−/− as compared with wt mice. The reduction in iNKT cell numbers in HVEM−/− mice was selective for the liver and not seen in spleen or blood (Fig. 2A, Table I). Reduced liver iNKT cell numbers in HVEM−/− mice may contribute to their mild course of ConA-induced hepatitis but does not explain the observed change in cytokine production. We asked if a change in phenotype of liver iNKT cells attenuates ConA-induced liver disease in HVEM−/− mice. Mitogen injection activated iNKT cells in vivo (up-regulated CD69 surface expression) and moderately enhanced their surface expression of CD80/CD86 costimulator and PD-1/PD-L1 (but not BTLA) coinhibitor molecules (Fig. 2B) in wt and HVEM−/− mice to a similar extent.

In contrast to the similar surface phenotypes of liver iNKT cells from wt and deficient animals, the cytokine expression profile differed. Liver NPC from HVEM−/− mice stimulated in vitro with titrated doses of either ConA, or the glycolipid αGalCer produced more IL-17 and IL-22 but similar amounts of IFN-γ and TNF-α (of all doses tested) than liver NPC from wt mice (Fig. 3). We did not detect IL-10, IL-27, or TGFβ in the supernatants of these cultures. We analyzed IL-17 and IL-22 expression of liver NPC populations from wt and HVEM−/− mice activated in vivo by i.v. ConA injection. Quantitative RT-PCR analyses showed up-regulated IL-17 and IL-22 transcript levels in HVEM−/− liver NPC populations harvested 14 h after ConA injection (Fig. 4). Ex vivo inducible release of IL-17 and IL-22 by liver NPC from mitogen-injected HVEM−/− mice was enhanced when compared with mitogen-injected wt mice (Fig. 4). Note that ConA stimulated enhanced IL-22 release by liver NPC from HVEM−/− mice more efficiently in vivo than in vitro (compare Figs. 3 and 4).

By intracellular cytokine staining ex vivo, liver iNKT cells obtained 2 h (Fig. 5A) or 16 h (Fig. 5B) after ConA injection from HVEM−/− mice produced less IFN-γ and IL-4, but more IL-22, than ConA-injected wt mice. No or very few IL-17-producing NKT cells or conventional CD4 T cells were detectable. We

### Table 1. Reduced iNKT cell population in the liver of HVEM−/− B6 mice

| Cells (×10^5) per mouse | Wild type | HVEM−/− | p value
|-------------------------|-----------|---------|--------
| Liver NPC               | 21.6 ± 0.22a | 18.3 ± 0.36 | 0.4766 |
| Liver non-NKT CD4 T cells | 1.57 ± 0.36 | 1.78 ± 0.27 | 0.6664 |
| Liver iNKT cells        | 2.71 ± 0.18  | 1.31 ± 0.22  | 0.0088 |
| iNKT cells in the liver NPC CD4 T cell population (%) | 51.60 ± 2.16 | 33.63 ± 3.07 | 0.0088 |

| Splenic iNKT cells | 1.30 ± 0.37 | 1.45 ± 0.21 | 0.7398 |

* p value < 0.05 is considered significant.

a Mean numbers of cells (×10^5) per tissue per mouse from five mice per group (±SEM).

b CD3+CD4+ T cells in the NPC population that do not bind CD1dαGalCer dimers.

c CD3+CD4+ T cells in the NPC population that bind CD1dαGalCer dimers.

d CD3+CD4+ T cells in the spleen that bind CD1dαGalCer dimers.

### FIGURE 3. In vitro cytokine responses of ConA- or αGalCer-stimulated liver NPC from wt and HVEM−/− mice. Liver NPC from wt and HVEM−/− mice were cultured for 16 h with or without the indicated doses of ConA or αGalCer. IL-17, IL-22, IFN-γ, and TNF-α release into supernatants was determined by ELISA. Mean values (±SD) from three independent experiments with two or three mice per dose per group.

### FIGURE 4. Cytokine expression of liver NPC from ConA-treated wt and HVEM−/− mice. RNA was isolated from liver NPC of wt and HVEM−/− mice 14 h after ConA injection. qRT-PCR was performed to detect IL-22 and IL-17 transcripts. The expression of B6 samples was set to one and values of treated animals are plotted as fold expression of the baseline (2−ΔΔCt). Bars represent the mean (±SD) fold enhancement of expression of the cytokine gene relative to hypoxanthine phosphoribosyltransferase or β-actin. The experiment was performed twice with similar results. Liver NPC from wt and HVEM−/− B6 mice injected 14 h previously with ConA were restimulated in vitro for 24 h with 2 µg/ml ConA. IL-17 and IL-22 levels were determined in supernatants by ELISA. Mean (±SD) cytokine levels from four mice per group (from two independent experiments) are shown.
thus found no evidence for a mitogen-activated CD4 Th17 response in the liver. Expression of IFN-γ, IL-4, or IL-22 by conventional, intrahepatic CD4<sup>high</sup> CD8<sup>low</sup> T cells was very low or undetectable in the course of this response (Fig. 5). ConA stimulation of liver iNKT cells from HVEM<sup>−/−</sup> mice in vivo thus generates more IL-22 producers but less IFN-γ and/or IL-4 producers than ConA stimulation of liver iNKT cells from wt control mice.

**HVEM-deficiency of liver NPC is associated with changes in the ConA-stimulated cytokine profile**

We found no differences in the IFN-γ, IL-4, IL-17, or IL-22 cytokine responses of purified, wt, or HVEM<sup>−/−</sup> liver iNKT cells cocultured for 18 h with purified, αGalCer-pulsed, wt, or HVEM<sup>−/−</sup> CD11c<sup>+</sup> hepatocytes (data not shown). It is thus unlikely that the different course of ConA-induced hepatitis in wt vs HVEM<sup>−/−</sup> mice results from differences in the iNKT cell/liver parenchyma interaction. This was confirmed using bone marrow chimeras (Fig. 6A). The course of ConA hepatitis was attenuated when the radiosensitive liver NPC population was HVEM-deficient but not when the radioresistant parenchymal cell population was HVEM-deficient.

ConA-stimulated activation of hepatitis-inducing iNKT cell responses is DC-dependent. This is apparent from the impaired induction of ConA hepatitis in transgenic CD11c<sup>DTR</sup> B6 mice in which CD11c<sup>high</sup> DC were depleted by diphtheria toxin (DT) (Fig. 6B). In vitro studies indicated that ConA-induced, enhanced IL-22 production by liver T cells is stimulated at the level of HVEM-deficient (non-CD4 T) liver NPC. Purified liver responder (CD3<sup>+</sup> CD4<sup>+</sup>) T cells from ConA-treated, wt, or HVEM<sup>−/−</sup> mice were cocultured with purified, CD4<sup>+</sup> T cell-depleted (CD4<sup>−</sup>) stimulator liver NPC from ConA-treated, wt, or HVEM<sup>−/−</sup> mice. Liver CD4<sup>+</sup> T cells from wt and HVEM<sup>−/−</sup> mice, cocultured with HVEM<sup>−/−</sup> (CD4<sup>−</sup>) liver NPC, produced more IL-22 but less IFN-γ than wt and HVEM<sup>−/−</sup> liver CD4<sup>+</sup> T cells cocultured with wt (CD4<sup>+</sup>) liver NPC (Fig. 6C, data not shown). HVEM expression by an intrahepatic, nonparenchymal stimulator cell population, but not by the responding CD4<sup>+</sup> T cell population, is thus critical for modulating the ConA-induced local cytokine profile of responding iNKT cells.

**Neutralization of IL-22 aggravates ConA-induced hepatitis in wt and HVEM<sup>−/−</sup> mice**

The course of ConA hepatitis is attenuated in HVEM<sup>−/−</sup> mice. Liver iNKT cells from HVEM<sup>−/−</sup> mice generate an enhanced IL-22 but attenuated IFN-γ response to i.v. ConA injection. To show that enhanced IL-22 production protects mice from ConA-induced hepatitis, we neutralized IL-22 by Ab injection at an early stage of disease induction. Eliminating IL-22 strikingly enhanced liver injury in ConA-treated, wt and HVEM<sup>−/−</sup> mice, evident by high serum transaminase levels (Fig. 7A) and more severe liver histopathology (data not shown). IL-22 neutralization furthermore down-modulated the serum IL-6 but up-regulated the serum IFN-γ response to ConA injection (Fig. 7B). The enhanced IL-22 responses in HVEM deficiency can thus contribute to the attenuation of the course of acute, T cell-dependent hepatitis elicited by mitogen injection. Anti-IL-22 Ab treatment of ConA-injected HVEM<sup>−/−</sup> mice increased their serum transaminase levels (i.e., aggravated liver injury) but this treatment did not reach the increased serum transaminase levels observed in anti-IL-22 Ab-treated, ConA-injected wt mice (Fig. 7A). IL-22-independent mechanisms may hence contribute to the protection from mitogen-induced acute hepatitis in HVEM<sup>−/−</sup> mice.
FIGURE 6. HVEM expression by stimulating liver NPC determines the severity of ConA hepatitis. A, Groups 1–4 bone marrow chimeras were constructed using wt CD45.1 or HVEM−/− CD45.2 donors and wt CD45.1, wt CD45.2, or HVEM−/− CD45.2 hosts. Reconstitution of the host with donor type lymphomyeloid cells was confirmed using CD45 (105/well) from HVEM−/− mice per group injected 16 h previously with allotype-specific Abs (with the exception of group 3). Mean serum ALT levels (±SD) of three chimeras per group injected 16 h previously with ConA (from one representative of two independent experiments) are shown. B, Wt and CD11c.DTR transgenic were treated with DT (5 ng/g body weight). This efficiently eliminated CD11c.DTR DC from the liver NPC population (tested 24 h and 48 h after DT injection). DT-treated wt or CD11c.DTR mice were injected i.v. with PBS (−) or ConA (+) and their serum ALT levels were measured 16 h later. Mean values (±SD) of three mice per group of one (of two independent) experiment are shown. C, CD4+ (responder) T cell and CD4+ (stimulator) cell populations were purified to >96% purity by MACS from liver NPC of HVEM+/+ and HVEM−/− B6 mice 14 h after ConA injection. Responder CD4+ T cells (105/well) from HVEM−/− (groups 1 and 4) or HVEM+/+ B6 mice (groups 2 and 3) were cocultured with stimulator CD4+ cells (105/well) from HVEM−/− (groups 1 and 2) or HVEM+/+ B6 mice (groups 3 and 4) for 16 h in the presence of 2 µg/ml ConA. Controls included 2 × 104 CD4+ T cells from HVEM−/− (group 5) or HVEM+/+ B6 mice (group 6), or 2 × 104 CD4+ T cells from HVEM−/− (group 7) or HVEM+/+ B6 mice (group 8) cultured in the presence of ConA. IL-22 in supernatants was determined by ELISA. Mean (±SD) IL-22 levels of triplicate cultures from three independent experiments are shown. n.s., Not significant. **, p < 0.005 show statistically significant differences.

Discussion

The severity of liver injury in ConA hepatitis is regulated by the balance between aggressive and protective cytokines. TNF-α, IFN-γ, and IL-4 promote liver inflammation, which is attenuated by IL-6, IL-10, and IL-22. Support is provided for the notion that liver iNKT cells are IL-22 producers and that HVEM expression by stimulating liver NPC down-modulates protective IL-22 responses in ConA hepatitis. HVEM on APC can hence promote proinflammatory responses, thereby aggravating T cell-dependent immunopathology in the liver.

The presented data are in conflict with a recent report that ConA hepatitis is more severe in HVEM−/− than wt mice (18). This report described high, systemic, proinflammatory cytokine responses but low serum transaminase responses in mitogen-injected HVEM−/− mice associated with high mortality within
hours after mitogen injection. We used 8–10-wk-old mice in this study. Mice >15-wk-old were used in the previous report. We found that 5-mo-old HVEM+/− mice contain an expanded population of T blasts in secondary lymphoid organs that show enhanced proinflammatory cytokine expression after ConA injection (data not shown). In older HVEM+/− mice, a mitogen-triggered, systemic cytokine storm with bystander hepatitis possibly trigger death associated with liver injury. This differs from the experimental model of ConA hepatitis that we investigated.

HVEM-deficient mice harbor low iNKT cell numbers in the liver but not other tissues. Impaired iNKT cell survival in the liver seems unlikely because we found similar numbers of annexin V+ (apoptotic) liver iNKT cells in nontreated or ConA-injected, wt, or HVEM+/− mice (data not shown). Deficient iNKT cell recruitment into the liver in HVEM deficiency may play a role. In addition to their numeric deficiency, intrahepatic iNKT cells from wt and HVEM+/− mice differed in their inducible cytokine profile. Liver iNKT cells from HVEM−/− mice produced more IL-22 in vivo after ConA injection or in vitro after αGalCer stimulation than liver iNKT cells from wt mice. Other data on in vitro mitogen/glycolipid-induced cytokine responses of wt and HVEM−/− mice did not correlate with the corresponding data on in vivo induced cytokine responses. IL-6 responses were reduced in the serum of ConA-injected. HVEM-deficient mice (Fig. 7B). IFN-γ responses were strikingly reduced in serum (Fig. 1B) and liver NKT cells (Fig. 5) in HVEM-deficient mice early and late after ConA injection, but no difference was seen in IFN-γ responses of liver NPC from wt and HVEM-deficient mice stimulated in vitro with ConA or αGalCer (Fig. 3). Recruitment of regulator and/or effector cells may operate in vivo that limit these IFN-γ responses in vivo. We consider the in vivo data more informative than the in vitro data.

The data show that liver NKT cells, but not conventional liver CD4 T cells, are the main IL-22 producers. In addition to NKT cells, the contribution of minor NPC subsets to the IL-22 response cannot be excluded. IL-22+ NKT cells do not qualify as Th17 cells, as only few of these cells coexpress IL-17 but many coexpress IL-4 and IFN-γ. Conventional CD4 Th17 cells were not detected in the liver under the experimental conditions used. Although IL-17 can play a pathogenic role in autoimmune diseases such as arthritis, dermatitis, or encephalitis (20), it does not seem to be involved in the proinflammatory responses of ConA hepatitis, as this experimentally induced hepatitis is similar in wt and IL-17−/− mice (16). Th17 cells (23) and other cells of the immune system produce IL-22, and IL-22 elicits responses that are independent of IL-17. One of these is the hepatoprotective (disease-attenuating) role of IL-22 in ConA hepatitis (16) that is confirmed by our IL-22 neutralization experiments.

iNKT cells trigger hepatitis after ConA injection but it is uncertain how the mitogen locally activates iNKT cells shortly after injection. ConA activation of T cells is an APC-dependent process (24). A difference in the induced cytokine profile was seen when CD4 T cells (including iNKT cells) were cocultured with CD4+ liver NPC from ConA-treated mice. The APC type that presents ConA in the liver in this system is not defined. The DC-depletion experiments described suggest that these professional APC are an attractive candidate. Intrahepatic DC express HVEM on the surface (data not shown). The described data indicate that HVEM on the surface of nonparenchymal liver APC modulate the cytokine response of iNKT cells. Unlike conventional T cells, iNKT cells activate IL-4 and IFN-γ transcription during thymic development, populate the periphery as long-lived effector cells with both cytokine loci constitutively active and rapidly secrete abundant amounts of cytokines only briefly after activation (25). iNKT cells are thus not irreversibly committed to a particular polarization but can coexpress Th1, Th2, and Th17 cytokines. Stimulation of iNKT cells with different (professional or nonprofessional) APC recalls different cytokine expression profiles. Apparently, HVEM on APC is an important regulator of the cytokine recall response of iNKT cells. The conflicting data of iNKT cells as suppressor or helper cells suggests that different APC displaying different costimulator/co-inhibitor expression profiles recall different functional phenotypes of iNKT cells. In the ConA hepatitis model, the absence of HVEM on liver APC favors local activation of hepatoprotective iNKT cells, and its presence favors activation of hepatitis-promoting iNKT cells. These checkpoints for the regulation of local, T cell-mediated inflammatory reactions may represent novel targets for the immune intervention in local, T cell-mediated immunopathology.

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Disclosures

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