Aryl hydrocarbon receptor plays protective roles in ConA-induced hepatic injury by both suppressing IFN-γ expression and inducing IL-22

Hiromi Abe 1–4, Akihiro Kimura 1, Sanae Tsuruta 1, Tomohiro Fukaya 1, Ryota Sakaguchi 1, Rimpei Morita 1, Takashi Sekiya 1, Takashi Shichita 1, Kazuaki Chayama 4, Yoshiaki Fujii-Kuriyama 5 and Akihiko Yoshimura 1

1Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo 160-8582, Japan
2Japan Science and Technology Agency (JST), CREST, Chiyoda-ku, Tokyo 102-0075, Japan
3Center for Medical Specialist Graduate Education and Research, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima 734-8553, Japan
4Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima 734-8551, Japan
5Medical Genomics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-8510, Japan

Correspondence to: A. Yoshimura; E-mail: yoshimura@a6.keio.jp

Received 1 April 2013, accepted 3 September 2013

Abstract

The aryl hydrocarbon receptor (AhR), a ligand-activated nuclear transcription factor, is known to mediate the toxic and carcinogenic effects of various environmental pollutants, while AhR has been shown to protect animals from various types of tissue injury. ConA-induced hepatitis is known as a mouse model of acute liver injury. Here, we found a protective role of AhR in ConA-induced hepatitis. AhR is induced in the liver during ConA-induced hepatitis, and Ahr−/− mice were highly sensitive to this model. Bone marrow chimera experiments indicate that Ahr−/− hematopoietic cells are responsible for hypersensitivity to ConA-induced hepatitis. We found that IFN-γ from invariant NKT cells was up-regulated and IL-22 from innate lymphoid cells (ILCs) was abolished in Ahr−/− mice. In addition, IL-22 production was still observed in Rag2−/− mice but it was severely reduced in Ahr−/−Rag2−/− mice. ConA-induced IL-22 production was also dependent on retinoic acid-related orphan receptor γt. These results show that AhR has crucial protective roles in ConA-induced liver injury via promoting IL-22 production from ILCs and suppressing IFN-γ expression from NKT cells.

Keywords: AhR, hepatitis, IFN-γ, IL-22, innate lymphoid cells

Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-inducible nuclear transcription factor and member of the Per-Arnt-Sim superfamily protein (1). The AhR is expressed ubiquitously and the expression levels are especially high in the lungs and the liver. AhR up-regulates the expression levels of cytochrome P-450 proteins (2). AhR and AhR nuclear translocator (ARNT) form a dimerization complex that works as a transcriptional locator in the nucleus (3). The complex participates in multiple regulatory mechanisms in response to xenobiotic metabolites, inflammation and cell cycling (2, 4). After nuclear translocation, the AhR–ARNT complex is regulated by ubiquitin-proteosomal degradation (5). AhR has been shown to regulate immune responses and tissue injury. For example, Ahr−/− mice have been shown to be resistant to experimental autoimmune encephalomyelitis and collagen-induced arthritis models (2–6), while sensitive to LPS-induced septic shock (7) and acute pancreatitis (8).

Innate lymphoid cells (ILCs) are known to have protective roles in innate immune responses to infection, repair after tissue damage, lymphoid tissue formation and homeostasis (6–8). ILCs lack re-arranged antigen receptors and act as a bridge between innate immunity and adaptive immunity (6). NK cells, which have been known as canonical innate lymphocytes, produce IFN-γ by sensing virus infection as well as Th1 cells. Recently, lymphoid tissue inducer cells that produce IL-17 and/or IL-22 have been shown to be involved in tissue remodeling and immunity. These cells, which express and require retinoic acid-related orphan receptor (ROR) γt and IL-7 for their development, can produce...
IL-22 (9, 10). IL-22 is a member of the IL-10 cytokine family and has crucial roles in epithelial homeostasis and antimicrobial defense in the gut (11). Recent reports showed that AhR regulates gut immunity through the modulation of ILCs (12, 13). It has also been reported that AhR-deficient mice lack cryptopatches and the IL-22 production by Rorγt+ ILC22 cells is significantly reduced (11).

It has been known that intravenous administration of ConA results in severe liver damage (14–18). ConA-induced liver injury has also been known to be dependent on activated T cells as well as NK cells (13, 16, 17). IL-17 has been reported to have a crucial role in ConA-induced liver injury (19–21), however, it has been reported that IL-22, but not IL-17, protects hepatocytes in ConA-induced liver injury (22). Two types of cells, namely CD4+ T cells and ILC22 cells, have been reported to produce IL-22. IL-6 is shown to be necessary for IL-22 production by Th22 cells (23). Dendritic cell (DC)-derived IL-23 has also been reported to be necessary for the production of IL-22 by ILC22 cells. Recently, Xu et al. (24) have shown that IL-23p19-deficient mice, but not IL-17-deficient mice, exacerbated ConA-induced liver cell injury along with a reduction of IL-22 levels, suggesting that IL-22 is produced from ILC22 and/or Th22 cells. IL-17, protects hepatocytes in ConA-induced liver injury (22).

In this study, we investigate the roles of AhR in ConA-induced hepatitis by analyzing the regulation mechanisms of cytokines. Our results show that Ahr−/− mice were highly susceptible to ConA-induced liver injury. The IFN-γ production level in the hepatic mononuclear cells (HMNCs) was higher but the IL-22 production in Ahr−/− mice was lower than that of wild-type (WT) mice. We found that IL-22 was mostly from ILCs in the liver of ConA-administered mice. These results suggest that AhR plays an important role in balancing IFN-γ and IL-22 production and protects the liver from acute liver injury.

Methods

Animals

AhR-knockout (KO) mice were provided from Y.F.-K. as described (7). Rag2−/− mice were from Jackson laboratory (25) and RORγt-GFP mice from Dr Littman (26). Ahr and Rag2 double KO (DKO) mice were generated by intercrossing. All mice were C57BL/6J background and maintained in specific pathogen-free conditions. All experiments using mice were carried out in accordance with the guidelines for animal care and use approved by Keio University.

ConA-induced liver injury

ConA-induced hepatitis was carried out as described (18). The mice were given a single intravenous injection of ConA (Sigma-Aldrich, St Louis, MO, USA) in saline at 10 μg g−1 of mouse body weight. The alanine transaminase (ALT) and aspartate transaminase (AST) activities in serum were measured by Oriental Yeast Co., Ltd. Recombinant Murine IL-22 (Peprotech) (2 μg per mouse) was mixed with ConA/saline then injected intravenously (24). Liver damage was determined by measuring the serum activities ofaminotransaminases (ALT and AST) (27) and H&E staining of liver sections. For histology, the liver tissues were fixed in 4% (w/v) paraformaldehyde. Fixed tissues were embedded in paraffin, then cut into 5-μm sections and stained with H&E.

HMNC preparation

HMNCs were isolated as described (28). Briefly, livers were removed from mice under diethyl ether anesthesia. After perfusing, the liver was minced and passed through a stainless steel mesh. The dissociated cells were washed with PBS, resuspended in an isotonic 37% Percoll solution (Amersham Biosciences, Arlington Heights, IL, USA) containing heparin [100 U ml−1 (Sigma-Aldrich)], and centrifuged at 2000 r.p.m. (500 × g) for 15 min at room temperature. The pellet was re-suspended in RBC lysis buffer and then was washed twice in RPMI 1640 medium supplemented with 10% fetal bovine serum.

Isolation and culture of NKT cells

NKT cells were isolated from HMNCs in WT mice using an NK1.1+ invariant NKT (iNKT) Cell Isolation Kit according to the manufacturer’s instructions (Miltenyi). The purity of NK1.1+ iNKT cells was consistently >95%. The NK1.1+ iNKT cells were stimulated with anti-CD3e (145.2C11, 5 μg ml−1), AhR agonist, 6-Formylindolo(3,2-b) carbazole (FICZ) (Enzo Life Sciences Inc.) and AhR antagonist, 2-Methyl-2H-pyrazole-3-carboxylic acid-(2-methyl-4-0-tolyl-azophenyl)-amide (CH-223191) (Merck) were dissolved with dimethyl sulfoxide and added into RPMI 1640 culture medium.

RNA extraction and quantification

RNA was extracted from mouse liver samples using RNeasy Mini Kit (QIAGEN, Valencia, CA), dissolved in RNase-free water and reverse transcribed using a random primer and M-MLV reverse transcriptase (Life technologies, Grand Island, NY) in a 25 μl reaction mixture according to the instructions provided by the manufacturer. One milliliter of cDNA was subjected to quantification by CFX96 (Bio-Rad, Hercules, CA) using the following primers: G3pdh, 5′-AACTTTGGCATTGTGGAAGG-3′ (sense) and 5′-GGATGCGAGGATGATTCTT-3′ (anti-sense); il22, 5′-TCAGCTCAGCTCTGCACTCATA-3′ (sense) and 5′-CCC AATCGCCTTGATCTCTCCA-3′ (anti-sense); Ahr, 5′-TTC TGA CGCTTCCACTACCCA-3′ (sense) and 5′-GGCTTCGT CCACCTCCTGT-3′ (anti-sense).

Flow cytometry

FACS analysis and cell sorting were performed as described (29, 30). The cells were incubated with each specific fluorescence-labeled mAb at 4°C for 30 min. Flow cytometry was performed using the following mAbs: anti-mouse anti-CD3 (PE-cy7), anti-NK1.1 (FITC), anti-Thy1 (APC) and anti-Sca1 (FITC). The cells were analyzed on FACSCan™ II (BD) and the data were analyzed by Flowjo software (Tree Star, Ashland, OR, USA).

In vitro stimulation of HMNCs and ELISA

HMNCs (1 × 106 per ml) were stimulated with PMA (50 ng ml−1) and ionomycin (1 μg ml−1) or Recombinant Murine IL-23 (R&D Systems) (40 ng ml−1) at 37°C for 5 or 5–24 h. NKT cells (1 × 105 per ml) were stimulated with ConA (10 μg ml−1) at 37°C for 5 or 72 h. The concentrations of IFN-γ and IL-22 in culture supernatants were measured with ELISA (eBiosciences, San Diego, CA, USA) as described previously (31, 32).
Fig. 1. *Ahr*−/− mice are susceptible to ConA. (A) Survival rate of WT mice (triangles) and *Ahr*−/− mice (squares) after ConA injection. (B) Serum ALT and AST levels 20 h after ConA injection. Bars indicate means ± SD (three mice per group); *P < 0.05 compared with WT mice. (C) Twenty-four hours after ConA injection, livers were stained with H&E. (D) Serum IFN-γ and IL-22 levels 12 h after ConA injection. Bars indicate means ± SD (three mice per group). (E) After ConA injection, the livers of WT and *Ahr*−/− mice (three mice per group) were prepared at indicated times and subjected to real-time PCR for *Ahr* mRNA expression (top) and protein analysis (bottom). The *Ahr* mRNA expression level was normalized to the transcript levels of *G3pdh*. Data are representative of two independent experiments.
Protein analysis

For immunoprecipitation, 20 mg of whole livers were lysed in lysis buffer [150 mM NaCl, 1% NP-40, 5 mM Tris (pH 8.0) added with protease inhibitor cocktail (Nacalai Tesque)] at 4°C for 1 h. The lysates were incubated with 1 μg of rabbit polyclonal antibody to mouse AhR (Enzo Life Sciences Inc.) at 4°C for 1 h and then incubated with 50 μl of protein A Sepharose (GE Healthcare). After the immunoprecipitates were washed with wash buffer [150 mM NaCl, 0.05% Tween 20, 5 mM Tris (pH 8.0)], proteins were dissolved with SDS polyacrylamide gel sample buffer, separated by SDS–PAGE and transferred to polyvinylidene fluoride membrane (Millipore). The membranes were incubated with rabbit polyclonal antibody to mouse AhR (1 μg ml⁻¹) (Enzo Life Sciences Inc.) and detected with the ECL system (GE Healthcare) (31).

Statistical analysis

Statistical analysis was performed by Student’s t-test and a P value <0.05 was judged as significant.

Results

Ahr⁻/⁻ mice are highly sensitive to ConA-induced hepatitis

First, to investigate the role of AhR in the liver injury, we injected ConA into Ahr⁻/⁻ mice. As shown in Fig. 1(A), all Ahr⁻/⁻ mice died within 24 h after ConA injection, while 100% of WT mice survived after 24 h. Serum ALT and AST levels 20 h after ConA injection were significantly higher in Ahr⁻/⁻ mice than in WT mice (Fig. 1B). Histological examination of the liver confirmed that Ahr⁻/⁻ mice appeared to suffer from very severe hepatic injury compared with WT mice (Fig. 1C).

Then, we compared serum IFN-γ and IL-22 levels (Fig. 1D). IFN-γ levels in Ahr⁻/⁻ mice were much higher than that in WT mice. In contrast, IL-22 levels in Ahr⁻/⁻ mice were lower than those in WT mice (Fig. 1D), which is consistent with more severe liver injury in Ahr⁻/⁻ mice than in WT mice. We also analyzed the expression levels of AhR by RT–PCR and western blotting after ConA administration (Fig. 1E). We observed rapid induction of AhR by ConA injection in WT mice liver. These data indicate that AhR is induced by ConA administration and functions protectively against T-cell- and NKT-cell-mediated liver injury.

Fig. 2. The effect of deficiency of AhR in hematopoietic cells. (A) Serum ALT and AST levels 20 h after ConA injection. Results are presented as means ± SD (five mice per group). (B) Serum IFN-γ and IL-22 levels 20 h after ConA injection. Bars indicate means ± SD (three mice for each group). **P < 0.01 compared with WT BM→WT. Data are representative of two independent experiments.
It has been reported that AhR is expressed in both hepatocytes and hematopoietic cells. To determine which AhR, in either hepatocytes or hematopoietic cells, is important for ConA sensitivity, we established bone marrow (BM) chimeric mice by transferring BM cells isolated from either Ahr<sup>+/+</sup> or Ahr<sup>−/−</sup> mice into lethally irradiated WT mice. Because of the limited number of available Ahr<sup>−/−</sup> mice and weakness of Ahr<sup>−/−</sup> mice following irradiation, we failed to reconstitute WT or Ahr<sup>−/−</sup> BM cells in Ahr<sup>−/−</sup> recipients. As shown in Fig. 2(A and B), ALT, AST and IFN-γ levels in mice that received a transfer of Ahr<sup>−/−</sup> BM cells were higher than in those of WT BM cells. In contrast, IL-22 levels in mice that received Ahr<sup>−/−</sup> BM cells were much higher than those with WT BM cells (Fig. 2B). These results indicated that a defect of AhR in hematopoietic cells caused promotion of IFN-γ production and suppression of IL-22 production after ConA administration.

**Enhanced IFN-γ production from Ahr-deficient NKT cells**

Next, we characterized IFN-γ-producing cells in ConA-induced hepatitis in Ahr<sup>−/−</sup> mice. It has been shown that T and NKT cells were major source of IFN-γ in ConA-induced hepatitis in WT mice (27). FACS analysis revealed that there were no significant differences in CD3<sup>+</sup> cell and NK1.1<sup>+</sup> cell populations in the HMNCs between WT and Ahr<sup>−/−</sup> mice (Fig. 3A). We observed a slight increase of the absolute number of NKT cells in Ahr-KO mice, however, this was not statistically significant. These data indicate that the NKT cell population and its activation by ConA were not affected by AhR deficiency.

![Fig. 3.](image-url) The IFN-γ production level in Ahr<sup>−/−</sup> mice is higher than that in WT mice. Mice were injected intravenously with 10 μg g<sup>−1</sup> of ConA. HMNCs were isolated 1.5h after ConA injection, stimulated with PMA (50 ng ml<sup>−1</sup>) and ionomycin (1 μg ml<sup>−1</sup>) for 5h. (A) CD3 and NK1.1 expression in HMNCs were analyzed by flow cytometry (three mice for each group). Representative figures are shown in the upper left of the figure and actual cell number counts were compared (right panel). (B–D) IFN-γ levels in the NKT cell culture supernatant measured by ELISA. (B) NKT cell were isolated from WT or Ahr<sup>−/−</sup> mice after ConA administration, then stimulated with PMA (50 ng ml<sup>−1</sup>) and ionomycin (1 μg ml<sup>−1</sup>) for 5h. (C) Hepatic NKT cells were isolated from HMNCs and then stimulated with 10 μg ml<sup>−1</sup> of ConA for 5h. (D) Hepatic NKT cells were isolated from HMNCs in WT mice and cultured with FICZ (300nM) or CH-223191 (3 μM) for 72h. NKT cells were pooled from five mice. The cells were stimulated with 5 μg ml<sup>−1</sup> anti-CD3ε antibody. Data are representative of two independent experiments.
Then, we examined IFN-γ production from NKT cells. We isolated NKT cell after ConA administration. IFN-γ production was much higher in cells from Ahr−/− mice than from WT mice (Fig. 3B). Then, we have isolated NKT cells from untreated mice and stimulated them with ConA in vitro. A shown in Fig. 3C, in vitro stimulation of isolated NKT cells also resulted in higher IFN-γ production from Ahr−/− NKT than from WT NKT cells. Then, we examined the effect of the AhR agonist FICZ and the synthetic antagonist CH-213191 on IFN-γ production from NKT cells (Fig. 3D). AhR should be activated by various compounds in the serum or medium (33). As shown in Fig. 3D, CH-213191 was ineffective in Ahr-deficient NKT cells, indicating that the effect of CH-213191 was Ahr-dependent. On the other hand, FICZ still suppressed IFN-γ production in Akr−/− NKT cells, suggesting that the effect of FICZ was Ahr-independent. FICZ may have other target molecules for regulating IFN-γ production from NKT cells. In any case, these data suggest that AhR plays an inhibitory role in IFN-γ production from T cells and NKT cells.

Detection of IL-22-producing ILC in HMNCs

It has been shown that IL-22 plays a protective role in the liver injury induced by ConA (23). To address whether severe liver injury was due to the reduction of IL-22 in ConA-treated Ahr−/− mice, we injected ConA together with recombinant IL-22 into Ahr−/− mice (Fig. 4). ConA-induced up-regulation of ALT and AST in Ahr−/− mice was drastically reduced by IL-22 administration (Fig. 4A). Interestingly, the IFN-γ in the serum of Ahr−/− mice was also reduced to the levels of WT mice by IL-22 (Fig. 4B). These data support the notion that IL-22 levels, at least in part, determined the severity of ConA-induced hepatitis in Ahr−/− mice.

Although IL-22 is shown to be protective for ConA-induced hepatitis, it has not been clarified whether similar ILCs are present in the liver or not. To make the contribution of ILC22 and T,22 cells for IL-22 production clearer, we used Ahr/Rag2 DKO mice, which lacked T,22 cells. Although Rag2−/− was highly resistant to ConA-induced hepatitis due to a lack of NKT and T cells, two out of three Ahr/Rag2 DKO mice died after ConA administration, suggesting that Ahr deficiency caused enhanced sensitivity to ConA even in the absence of T cells (data not shown).

Then, we examined IL-22 mRNA expression in CD3−Sca1+Thy1+ cells (ILC fraction) and CD3+ T cells in WT, Ahr−/− and Ahr−/−Rag2−/− mice after sorting by FACS (Fig. 5A). Cells were stimulated by IL-23 to induce IL-22 expression. IL-22 mRNA levels were high in CD3−Sca1+Thy1+ cells rather than CD3+ T cells, suggesting that the major source of IL-22 in

---

**Fig. 4.** AhR is important for IL-22 production in ConA-induced hepatitis. Serum ALT, AST (A) and IFN-γ (B) levels 8 h after injection of ConA (10 μg g−1 of mouse body weight) or ConA plus IL-22 (2 μg per mouse). Bars are means ± SD (three mice per group); *P < 0.05 compared with Ahr−/− mice that were injected with only ConA. Data are representative of two independent experiments.
Fig. 5. CD3−Sca1+Thy1+ innate lymphoid cells are major producers of IL-22 in the liver of ConA-administrated mice. (A) Mice were injected intravenously with 10 μg g−1 of ConA. HMNCs were isolated 1.5 h after Con A injection and stimulated with IL-23 (40 ng ml−1) for 18 h. Sca1 and Thy1 expression were analyzed after gating on CD3− cells by flow cytometry. Representative data of three independent experiments of WT mice are shown. Similar FACS profiles were obtained for Ahr−/− and Ahr−/−Rag2−/− mice. (B) CD3+ cells and CD3−Sca1+Thy1+ cells were sorted by FACS from IL-23-stimulated (+) or unstimulated (−) HMNCs from WT, Ahr−/− and Ahr−/−Rag2−/− mice treated with ConA. The Il22 mRNA expression level was normalized to the transcript levels of G3pdh. *P < 0.05. (C) IL-22 levels were measured by ELISA in the culture supernatant of HMNCs isolated from indicated mice treated with ConA. HMNCs were stimulated with PMA (50 ng ml−1) and ionomycin (1 g ml−1) for 24 h. Bars are means ± SD (three mice for each group). *P < 0.05 Data are representative of two independent experiments. (D) Serum IFN-γ and IL-22 levels in WT and Rorgt−/− mice 8 h after ConA injection. Bars indicate means ± SD (three mice for each group), *P < 0.05.
the liver was ILC22 rather than T_{h}22 cells (Fig. 5B). Although the CD3−Sca1−Thy1+ fraction was present in Ahr−/− and Ahr/Rag2DKO mice, Il22 was undetectable in these cells (Fig. 5B).

This was confirmed by ELISA detection of IL-22 from HMNCs (Fig. 5C). As shown in Fig. 5(C), almost no IL-22 production was observed in the HMNCs of Ahr−/− as well as Ahr−/−Rag−/− mice.

To confirm that ILC22 was a major IL-22-producing cell type in the liver, we used RORγt-deficient mice, since it has been reported that RORγt is essential for the development of ILC22 cells (33). As shown in Fig. 5(D), like Ahr−/− mice, RORγt-deficient mice produced no detectable IL-22 after ConA administration, while these mice produced higher levels of IFN-γ than WT mice. Similarly, no IL-22 production was observed in the HMNCs of RORγt-deficient mice (see Fig. 5C). These data support our proposal that IL-22 was produced from ILC22 but not T_{h}22 cells in the liver.

Discussion

AhR has been reported to play an important role in the development of ILC22 cells in the gut (10–12). Previous reports showed that NKT cells abound in the liver and produce a large amount of IFN-γ after stimulation in the ConA-induced liver injury model (14, 15). Although it has been reported that IL-22 plays a protective role in ConA-induced liver injury as IL-22−/− mice develop more severe hepatitis than WT mice do (21), whether AhR also plays an important role in ConA-induced liver injury is unknown. Accordingly, we investigated in this study the role of AhR on production of IL-22 in ConA-induced liver damage using Ahr−/− mice. All Ahr−/− mice that were injected with ConA died within 24h in contrast to WT where all treated mice survived. IL-22 production levels in these Ahr−/− mice were very low (Figs 1 and 2). In contrast, the serum levels of IFN-γ were higher in Ahr−/− mice than WT (Fig. 1D). High IFN-γ levels were also observed in mice with replace Ahr−/− BM, showing that presence of AhR in blood cells is important for AhR to play a protective role. This is in contrast to the results of IL-22 KO mice where production of IFN-γ is almost comparable between WT and IL-22-deficient mice. Therefore, AhR has two protective roles in ConA-induced liver injury, namely induction of IL-22 production and repression of IFN-γ secretion. Our results showed that the population of cells in the liver after ConA administration is not different between WT and Ahr−/− mice (Fig. 3). This suggests that AhR reduces IFN-γ in the cells without altering proliferation and infiltration levels of NKT cells in the liver (Fig. 3).

Although it is assumed that the increased serum IL-22 levels after ConA injection seen in WT mice might come from intestinal ILC22, whether proliferation and infiltration of ILC22 cells in the liver after ConA injection occurs is unknown. We thus attempted to detect ILC22 cells in the liver. We observed the ILC fraction in WT HMNCs (Fig. 5). These results suggest that ILC22 cells are induced at the site of inflammation and had an important role to control inflammatory activity in the organ. Administration of recombinant IL-22 confirmed the protective effect of this cytokine in this model (Fig. 4). We further analyzed the IFN-γ and IL-22 production levels in the mononuclear cells in the small intestinal lamina propria and the Peyer’s patches after injection of ConA into WT mice. We could detect neither IFN-γ nor IL-22 (data not shown). It has also been reported that CD4+ T cells produce IL-22 in splenocytes after anti-CD3 stimulation (22). However, we could not detect IL-22 in CD4+ T cells in splenocytes and HMNCs after injection of ConA into even WT mice (data not shown). Our results showed both the high level of IFN-γ and the low level of IL-22 cause severe liver injury in Ahr−/− mice injected with ConA.

Interestingly, ALT and AST elevation and IFN-γ levels in serum samples became lower when we administered recombinant IL-22 to Ahr−/− mice. This is in contrast to the finding that IFN-γ levels were not significantly changed in IL-22-deficient mice (23). This may be because in the WT situation, IL-22 is induced by IL-23 from macrophages or DCs, which is activated by IFN-γ or other factors after NKT cell activation. Since exogenous IL-22 strongly suppresses liver cell damages, further IFN-γ production from T cells or NKT cells may be blocked.

Furthermore, IFN-γ production was enhanced by the AhR antagonist, CH-223191. These results suggest that there should be an interaction between AhR and T-bet, which plays essential roles in IFN-γ transcription in T cells. However, it is still not very clear how AhR increase IL-22. AhR could be essential for ILC22 development in the liver like in the intestine, or AhR might directly regulate IL-22 transcription. As reported, IL-22 was undetectable in RORγt-deficient mice, indicating that IL-22 production in ConA hepatitis is totally dependent on RORγt. In addition, recent paper showed that RORγt/Rag-DKO mice developed significantly severer CC14-induced hepatitis compared with Rag-KO mice, in accordance with reduced IL-22 production from hepatic ILCs (34). These data strongly suggest that IL-22 production from ILCs in the liver is dependent on AhR and RORγt. Further study is necessary to determine how AhR promotes IL-22 expression.

In conclusion, we showed that AhR protects liver cells through suppression of IFN-γ and induction of ILC22 cells in the liver. Further study is needed to make the unknown interaction between ILC22 cells, AhR and IFN-γ.

Funding

Ministry of Education, Culture, Sports, Science and Technology of Japan; the SENSIN Medical Research Foundation, the Mochida Memorial Foundation; the Uehara Memorial Foundation; KANAE foundation for the Promotion of Medical Science.; Astellas Foundation for Research on Metabolic Disorders The Ichiro Kanehara Foundation; the Takeda Science Foundation.

Acknowledgements

We thank R. Yoshida, N. Shino, N. Shimizu, Y. Yogiashi and M. Asakawa for technical assistance and Y. Ushijima for manuscript preparation.

Conflict of Interest statement: The authors have no conflicting financial interest.

References

Protective role of AhR in ConA-induced hepatitis