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Suppressive and pro-inflammatory roles for IL-4 in the pathogenesis of experimental drug-induced liver injury: a review

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Abstract

Importance of the field—Idiosyncratic drug reactions resulting in drug-induced liver injury (DILI) account for ~ 13% of acute liver failure cases in the US. Idiosyncratic drug reactions are the third most common cause of liver transplantation, exceeded only by acetaminophen and indeterminate causes. Clinical evidence suggests that idiosyncratic DILI is triggered by drug hapten-altered self proteins resulting in hepatocellular injury. An example of this type of DILI is hepatitis that develops in susceptible individuals following administration of halogenated volatile anesthetics, dihydralazine, carbamazepine or diclofenac.

Areas covered in this review—In this review, we describe research in animal models that supports a critical role for suppressive and pro-inflammatory roles for IL-4 in the pathogenesis of immune-mediated DILI.

What the reader will gain—The reader will gain insights into the roles of IL-4 in the development of experimental DILI. The reader will gain tools to assist in the translation of these findings to those in patients with immune-mediated DILI, as well as other inflammatory diseases of the liver. The reader will then be made aware of gaps in knowledge in the pathogenesis of DILI where research could result in significant advances in the care of these complicated patients.

Take home message—In experimental immune-mediated DILI, IL-4 suppresses regulatory responses to CYP2E1 autoantigens but induces pro-inflammatory responses to drug haptens.

Keywords

CYP2E1; DILI; drug haptens; halogenated anesthetics; IL-4; trifluoroacetyl chloride

1. Introduction

Drug-induced liver injury (DILI) from idiosyncratic drug reactions accounts for ~ 13% of acute liver failure cases in the US [1,2] and represents the third largest cause of acute liver failure from all causes. Idiosyncratic DILI that follows the administration of halogenated volatile anesthetics, tienilic acid, dihydralazine, carbamazepine or diclofenac is believed to be immune-mediated [3,4]. Unfortunately, precise pathogenic mechanisms responsible for the initiation or subsequent development of hepatitis are not fully elucidated.

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Even so, it is generally accepted that immune responses are triggered by complex antigens produced from native hepatic proteins such as CYP enzymes [5] that have become covalently modified by drug haptens formed during the oxidative metabolism of the drug by these enzymes [6–8]. Immune responses occurring in secondary lymphoid organs generate cytokines that promote the development of inflammation in the liver [9,10]. Additionally, haptenated native liver proteins induce both anti-hapten antibodies, as well as autoantibodies to CYP450 enzymes [11–14]. In immune-mediated DILI, these antibodies may have a role in augmenting systemic or hepatic immune responses [10,15] or augmenting metabolic consequences of hepatic injury [13].

IL-4 is one cytokine that has been linked to the development of immune-mediated DILI as well as its associated antibodies. An earlier study has clearly associated variant IL-4 alleles with the development of immune-mediated DILI from diclofenac [16]. This landmark study is one of the earliest studies to directly associate abnormalities in IL-4 signaling or expression with the development of immune-mediated DILI in patients. In a later study, we have demonstrated CYP2E1 autoantibodies of the IgG4 subclass in the sera of people with halogenated volatile anesthetic DILI [15]. Detecting CYP2E1 autoantibodies of the IgG4 subclass strongly suggests a role for IL-4 in the development of these autoantibodies [17] and suggests that IL-4 promotes the development of immune-mediated DILI. Moreover, previous studies have clearly shown that IL-4 and CD4⁺ T cells can promote the development of allergic and autoimmune diseases [18,19] by inducing B-cell proliferation and isotype switching from IgM to IgG4 and IgE [17,20]. In people with immune-mediated DILI, these IgG4 autoantibodies can then form circulating, non-precipitating immune complexes that could damage hepatocytes [15,21], further supporting a critical role for IL-4 in propagating this disease.

We have formulated a model of the initiation of immune-mediated DILI from anesthetics [9]. Using this model, we have uncovered critical IL-4-dependent mechanisms supporting our notion for suppressive and pro-inflammatory roles for IL-4 in the development of anesthetic DILI [10]. More importantly, these mechanisms are similar to those seen in patients with immune-mediated, anesthetic DILI [14,15,21,22]. To our knowledge, this is the only model of immune-mediated DILI that directly demonstrates roles for drug haptens and autoimmune responses in its pathogenesis. In this review, we describe research in animal models that supports a critical role for suppressive and pro-inflammatory roles for IL-4 in immune-mediated DILI. We begin by demonstrating multifaceted roles for IL-4 in animal models of autoimmune diseases and in immune-mediated hepatitis. We translate findings in experimental DILI to DILI in patients as well as other inflammatory diseases of the liver. Then, we discuss gaps in knowledge in the pathogenesis of immune-mediated DILI where research could result in significant advances in the care of these complicated patients, as well as the care of patients with chronic hepatic inflammation from other etiologies.

2. The multifaceted roles of IL-4

IL-4 is a cytokine that most commonly represents T_H2 responses. However, analogous to a floating iceberg, this role is only what is seen above the surface while the bulk of the actions of IL-4 and even their significance are beneath the waterline. IL-4 is a multi-faceted cytokine with effects on diverse populations of cells. In these roles, depending on the cell type, IL-4 can induce regulatory, suppressive or even pro-inflammatory responses. Thus, because of its diverse population of resident non-immune and immune cells and even infiltrating immune cells in pathological conditions it should be no surprise that all of these responses come to roost in the liver. In this section, we initially describe cell populations responsive to IL-4. Then, we review IL-4 intracellular signaling pathways that are particularly relevant to immune-mediated DILI. Last, we review how IL-4 promotes CD4⁺

T-cell antigen recognition and describe how this may have a role in the pathogenesis of immune-mediated DILI.

2.1 IL-4 acts on diverse cell populations

IL-4 induces pro-inflammatory, suppressive and even regulatory responses on immune or non-immune cells. IL-4 can also promote the development of specific subtypes of immune cells that can produce more IL-4 (Figure 1). In this role, IL-4 promotes the proliferation of CD4⁺ T_H2 cells that produce IL-4, IL-5, IL-6 and IL-10 [23]; however, IL-4 also promotes the development of CD8⁺ T (T_C2) cells that also produce more of this cytokine. IL-4 demonstrates its regulatory roles by promoting differentiation and isotype switching of B cells, as well as promoting CYP2E1 expression by hepatocytes [24]. In addition, IL-4 modulates macrophage function by downregulating stimulatory Fc receptors and subsequently upregulating inhibitory FcγRII [25]. More recently, pivotal roles for IL-4 have been demonstrated in immunoregulation by NK and NKT cells. In these roles, IL-4 can downregulate IFN-γ expression by NK or NKT cells which could promote T_H2 priming and even tolerance to invading antigens [26,27]. Interestingly, mast cells and basophils, cells associated with autoimmune and allergic responses, also produce IL-4. Thus, because of multiple effects on different cell types and its capability of generating more IL-4 production, it is not surprising that IL-4 could have multiple roles in the development of experimental autoimmune diseases, experimental immune-mediated hepatitis and especially experimental immune-mediated DILI. Moreover, clinical evidence suggests that multifaceted roles for IL-4 may have a significant role in the pathogenesis of immune-mediated DILI in patients [15,16]. However, in order to assimilate the data supporting these roles for IL-4, we must first understand key components of IL-4 receptor signaling that could have an important role in the development of immune-mediated DILI.

2.2 IL-4 receptor engagement activates multiple intracellular signaling pathways

Vital to the actions of IL-4 is its ability to promote expression of genes and proteins responsive to this cytokine. Because of its well-recognized role in the development of autoimmunity and allergy, it comes as no surprise that MHC II, IgE as well as more IL-4 receptor α (IL-4Rα) are among the genes upregulated by IL-4. Intracellular signaling resulting in upregulation of these genes is initiated by IL-4 engagement of the IL-4 receptor, which is formed by the two side chains IL-4Rα as well as the IL-2Rγ chain (Figure 2) [28]. Next, a complex sequence of signals occurs which include tyrosine phosphorylation of JAK1, which is associated with the IL-4Rα chain, as well as JAK3 phosphorylation, which is associated with the IL-2Rγ chain. STAT6 then binds to the phosphorylated receptor allowing its phosphorylation by JAK1. The phosphorylated STAT6 dislodges from the receptor and forms homodimers that are disrupted through enzymatic action by STAT6 proteases. STAT6 is now capable of translocation to the cell nucleus where it binds to DNA and promotes transcription of MHC II, IL-4 and IL-4Rα (Figure 2). Obviously, these proteins can have a significant role in the development of immune responses to either drug haptens or autoantigens. Even so, we cannot exclude the well-known fact that IL-4 can also regulate cell growth and differentiation by signaling through the insulin receptor substrate1/2 or the Ras–MAP kinase signaling pathway. Unmistakably, because of its inherent complexity, in order to understand the critical role for IL-4 in this process, we must next review well-known roles of IL-4 in promoting T-cell antigen recognition by CD4⁺ T cells.

2.3 IL-4 promotes CD4⁺ T-cell antigen recognition

For successful generation of CD4⁺ T-cell effector function, the CD4⁺ T cell must first recognize the immunogenic antigen. Successful recognition of antigen then induces T-cell activation and subsequent CD4⁺ T-cell proliferation. CD4⁺ T-cell proliferation promotes the

development of immune responses to the immunogenic antigen in the form of immunosuppression, immune regulation, antibody formation or even organ-specific autoimmunity. Thus, reviewing the role of IL-4 in known mechanisms of successful antigen recognition by CD4⁺ T cells is crucial in understanding roles for IL-4 in mechanisms of organ-specific autoimmunity.

Successful antigen recognition by CD4⁺ T cells requires MHC II antigen presentation of the immunogenic peptide by professional antigen presenting cells, such as dendritic cells, B cells or macrophages [29]. Remember, IL-4 has well-known actions on both B cells and macrophages. Interestingly, mast cells and basophils, cells that have been associated with the development of allergy and autoimmunity, can also respond to IL-4 in their environment through MHC II upregulation and presentation of MHC II antigen complexes to CD4⁺ T cells. IL-4 accomplishes upregulation of MHC II expression using JAK1/STAT6 signaling pathways (Figure 2) which are regulated at least in part by the MHC II trans activating protein CIITA [30]. MHC II expression promotes expression of mature α and β chain MHC II molecules on T cells, as well as γ and δ molecules on some T cells.

In addition to promoting expression of specific MHC II molecules, IL-4-induced upregulation of MHC II is isotype specific [31], where IgG1 and more specifically IgG4 isotype antibodies are associated with this cytokine. Curiously, isotype specificity by IL-4 or even other cytokines may be supported by previous clinical studies where roles of IgG subclasses have been demonstrated in autoimmune diseases [32]. Moreover, IgG4 subclass specificity has been clearly described in organ-specific autoimmunity in Graves' disease [33], pemphigus [34], pancreatic autoimmunity [34] and even immune-mediated DILI from halogenated volatile anesthetics [15]. These studies firmly demonstrate multiple roles for IL-4 in the generation of CD4⁺ T-cell responses and begin to suggest that these mechanisms may have a crucial role in the development of organ-specific autoimmunity. With the background story of IL-4 briefly reviewed, we can begin to address multiple roles for IL-4 in autoimmunity by next reviewing well-known studies that describe the role of IL-4 in autoimmune disease models.

3. Roles for IL-4 in classical experimental autoimmune disease models

The pathogenesis of many experimental autoimmune disease models involves proliferation of autoreactive T cells supported by T_H1-mediated immune responses. Because of its known ability to downregulate T_H1 mediated responses, roles for IL-4 have been investigated in many well-known experimental models of autoimmune disease. From these investigations, it has been uncovered that IL-4 decreases inflammation in experimental autoimmune disease models such as autoimmune diabetes, experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA). In sharp contrast, IL-4 may have anti- or pro-inflammatory roles in experimental autoimmune myocarditis. In order to reconcile these findings, many investigators have determined that the role of IL-4 depends on the antigen used to induce autoimmunity, the strain of mouse and even the stage of the disease in the model being investigated. We show that even in time-honored experimental autoimmune disease models, there is evidence for multiple immunomodulatory roles for IL-4. We propose that these multifaceted roles can either induce pro- or anti-inflammatory pathways to achieve either up or downregulation of immune responses to autoantigens used to induce these animal models.

3.1 IL-4 in experimental diabetes

The most well-known spontaneous model of autoimmunity occurs in non-obese diabetic (NOD) mice. In this model, first developed in 1980, 60 – 90% of female mice will have glycosuria by the 10 – 12th week of life and ultimately insulin-dependent diabetes mellitus [35]. However, as early as 2 – 4 weeks of age, islet antigens can be detected in the draining

lymph nodes [36]. Insulin-dependent diabetes is also preceded by non-pathogenic autoantibodies against insulin similar to patients with this disease [37]. So NOD mice are widely used because of their translational similarities with patients who have insulin-dependent diabetes mellitus [38].

Experimental diabetes in NOD mice is commonly used to demonstrate anti-inflammatory roles for IL-4. In this classical example, IL-4 diminishes disease by directly reducing autoantigen-induced T_H1 immune responses in pancreatic β cells [39]; however, additional roles for IL-4 may be present in this model. One indirect role may involve the maturation of Foxp3⁺ CD25⁺ CD4⁺ regulatory T cells. In recent years, regulatory T cells have been suggested as agents for adoptive immunotherapy in autoimmune diseases [40]. With this in mind, previous studies have clearly shown that engagement of the IL-4 receptor by IL-4, or even its effector cytokine IL-13, is required for the maturation of Foxp3⁺ CD25⁺ CD4⁺ regulatory T cells from CD25-CD4⁺ T-cell precursors [41]. Hence, IL-4 expression would block the progression to diabetes in NOD mice by promoting the maturation of Foxp3⁺ regulatory T cells [42]. Moreover, these regulatory T cells could then release more IL-4 which could further block progression to clinical diabetes in these mice. Thus, in experimental diabetes in NOD mice IL-4 has two roles, one that is directly anti-inflammatory and an additional immunoregulatory role where IL-4 enhances the development of immunosuppressive Foxp3⁺ regulatory T cells.

3.2 IL-4 in experimental autoimmune encephalitis

In some instances, IL-4 may also decrease inflammation in models of experimental autoimmunity by inducing pro-inflammatory responses on a cellular level. In support of this statement, the decrease in EAE in Lewis rats treated with intranasal IL-4 and subsequently immunized with myelin basic protein 68 – 86 is caused by enhanced proliferation of dendritic cells expressing high levels of IFN- γ and IL-10 [43]. Further stimulation of these dendritic cells by IL-4 induces production of NO. Production of NO promotes apoptosis of autoantigen-induced, autoreactive T cells. Therefore, while the final result was a decrement in inflammation in EAE, IL-4 accomplishes this end point using pro-inflammatory pathways.

3.3 IL-4 in experimental collagen-induced arthritis

In CIA, dual roles for IL-4 have been suggested. In this model, autoimmune arthritis is induced by immunizing rodents with type II collagen emulsified in complete Freund's adjuvant on days 0 and 7. Arthritis then develops ~ 11 – 14 days after the second collagen immunization. In a landmark study, it has been clearly demonstrated that administration of continuous recombinant IL-4 attenuates CIA [44]. In support of this finding, a later study has clearly shown that the majority of IL-4 deficient mice on a C57Bl/10 background develop an attenuated course of CIA following immunization with collagen emulsified in incomplete Freund's adjuvant. However, this study has also shown that a small subset of these same mice developed a chronic relapsing form of CIA [19]. These findings have led the authors to conclude that the role of IL-4 may depend on the adjuvant used in experimental models as well as the stage of the disease. Remarkably, this conclusion was a daring venture for these authors because at the time these studies were described, it was generally accepted that IL-4 purely had an anti-inflammatory role in the pathogenesis of experimental organ-specific autoimmunity.

3.4 IL-4 in experimental autoimmune myocarditis

In experimental autoimmune myocarditis, multiple roles for IL-4 have also been demonstrated. In this model, IL-4 deficient A/J mice develop significantly less myocarditis when compared to wild-type mice following immunization with cardiac myosin

demonstrating a pro-inflammatory role for IL-4 in the development of myocarditis and antibodies [44]. In sharp contrast, a pro-inflammatory role for IL-4 is not observed when myocarditis is induced using a myocardiogenic peptide in IL-4 deficient mice on a BALB/c mice background [45]. In the latter IL-4 study, the authors have also shown that IL-13, the effector cytokine of IL-4, is clearly protective. Moreover, these authors have also determined that IL-13 is performing a protective role in myocarditis through modulating macrophage function in these mice. In the former study, IL-4 deficient mice are on an A/J background, suggesting that the contribution of the mouse strain must be taken into account in determining the role of IL-4 as has been demonstrated in acetaminophen toxicity where animal strain and susceptibility to injury have been recently analyzed [46]. These studies also serve as a reminder of the importance of the immunogen, cardiac myosin versus a myocardiogenic peptide, when determining the role for IL-4 in these models. More importantly, dual roles for IL-4 are unmistakably evident in long-standing, classical models of organ-specific autoimmunity (Table 1).

4. Pro-inflammatory roles for IL-4 in experimental immune-mediated hepatitis

Thus far, we have described studies where IL-4 may have a pro- or anti-inflammatory role in the development of organ-specific inflammation in classical autoimmune disease models. However, when investigating IL-4 in experimental immune-mediated hepatitis, a pro-inflammatory role is heavily supported. Previous studies have correlated IL-4 with the development of hepatitis following the administration of the plant lectin concanavalin A (Con A) and high dose lipopolysaccharide (LPS) to mice. So next, we describe the role of IL-4 in the development of T cell- and macrophage-mediated hepatitis, respectively.

4.1 IL-4 in experimental Con A-induced hepatitis

Con A administration to mice induces expression of multiple cytokines, including IL-4 and IFN- γ by activated T cells. The hepatitis that follows consists of neutrophils and eosinophils. A previous study has shown that IL-4 and Stat6 deficient mice develop significantly less neutrophilic and eosinophilic hepatic inflammation following Con A administration [47], demonstrating a role for IL-4 as well as its intracellular signaling pathways (Figure 2) [28] in the pathogenesis of Con A-induced hepatitis. This study concludes that IL-4 has a key role in Con A hepatitis through its upregulation of eotaxin in hepatocytes and hepatic sinusoidal cells as well as its induction of IL-5 expression which promotes recruitment of neutrophils and eosinophils to the liver. In support of these findings, a later study has shown that IL-4 in conjunction with CD4⁺ T cells induces liver injury by activating the Jak1/ Stat6 pathway (Figure 2) in hepatocytes and NKT cells. This activation also induces liver injury through the production of eotaxin and IL-5 [48]. Thus, these studies allow us to entertain the possibility that IL-4 and its intracellular signaling pathways may have a pro-inflammatory role in the development of hepatic inflammation initiated by T cells in addition to earlier studies demonstrating a pro-inflammatory role in cellular immune responses in response to certain autoantigens.

4.2 IL-4 in LPS-induced hepatitis

Interestingly, earlier studies have also suggested that IL-4 may have a key role in the development of high dose LPS-induced hepatitis. This is particularly intriguing because LPS- induced hepatitis is believed to occur primarily through activation of macrophages and not T cells. As early as 1 h and as late as 24 h following administration of high dose LPS to mice, significantly elevated levels of IL-4 have been detected in the plasma of these mice [49]. A subsequent study further supports this finding by describing a critical role for IL-18 in LPS-induced hepatitis. The association with IL-18 is an important finding because IL-18

is known to induce IL-4 expression in T_H cells which then activates mast cells and basophils in allergic responses [50].

We must not forget that while in 2010 we are not surprised by this role for IL-4 in macrophage activation, the suggestion that IL-4 may be involved in the activation of macrophages represented a huge paradigm shift in the understanding the pathogenesis of immune-mediated hepatitis. Moreover, these studies have clearly demonstrated that IL-4 activation of macrophages requires a strong stimulus, such as high dose LPS, because it was not demonstrated in another mouse model of macrophage hepatitis that develops following lower doses of LPS in mice sensitized with the amino-acid sugar D-galactosamine hydrochloride [49]. Hence in experimental immune-mediated hepatitis, whether induced by T-cell stimulation with Con A or macrophage stimulation with high dose LPS, IL-4 clearly has a pro-inflammatory role (Table 2).

5. Roles for IL-4 in experimental DILI

Thus far, we have presented evidence for immunomodulatory roles for IL-4 in response to autoantigens in models of organ-specific autoimmunity and in response to T-cell stimulants or endotoxin in models of immune-mediated hepatitis. To begin to uncover roles for IL-4 in pathogenesis of DILI, we need to acknowledge distinctive mechanisms that have a significant role in this process. It is generally accepted that DILI can occur from two mechanisms: direct toxicity from toxic drugs or their metabolites, or immune-mediated toxicity in response to drug hapten altered native proteins. Interestingly, in some cases of DILI, both mechanisms could have a role in the severity of hepatitis. Hence, DILI is a unique condition with complex responses to toxic metabolites or even drug haptens may have an impact on the role of IL-4.

In contrast to immune-mediated models of hepatitis, a pro-inflammatory role for IL-4 may not be consistently seen in models of DILI following administration of toxic chemicals. In hepatic injury and inflammation following carbon tetrachloride (CCL₄), for example, roles for IL-4 have not been sufficiently investigated; however, in hepatic injury following toxic doses of acetaminophen, experimental evidence suggests a clear pro-inflammatory role for IL-4. We have also recently demonstrated critical suppressive and pro-inflammatory roles for IL-4 in the development of immune-mediated DILI in an animal model of anesthetic DILI [10]. So next, we describe previous investigations on the role of IL-4 in DILI from toxicity following CCL₄ and acetaminophen, as well as our conclusions that IL-4 acts by suppressing regulatory cytokine responses to the CYP2E1 autoantigen and by inducing pro-inflammatory cytokine responses to trifluoroacetyl chloride (TFA) haptenated proteins in our model of immune-mediated DILI from halogenated anesthetics.

5.1 IL-4 in experimental DILI following carbon tetrachloride

The role of IL-4 is not commonly discussed in models of drug-induced hepatotoxicity; however, this fact may be supported by the multiple mechanisms required for this type of injury making interpretation of some of these studies a challenging enterprise. Even so, in a well-known model of CCL₄ hepatotoxicity, a direct role of IL-4 in the development of DILI or even fibrosis has been investigated [51]. Previous investigators have clearly shown that BALB/c mice develop more significant hepatic injury and fibrosis following administration of toxic doses of CCL₄ by gavage feeding when compared to C57Bl6/J mice. These authors elegantly confirm upregulation of hepatic IL-4 in CCL₄-treated BALB/c mice, as well as inhibition of hepatic fibrosis in BALB/c mice treated with intraperitoneal anti-IL-4 during CCL₄ feedings.

5.2 IL-4 in experimental DILI following acetaminophen

In acetaminophen-induced hepatotoxicity, IL-4 has clearly been shown to have a pro-inflammatory role in the development of hepatitis. Unfortunately, similar to CCL4, it is not a topic that is commonly discussed. Nevertheless, an elegant study by Bourdi *et al.* has clearly demonstrated that IL-4 deficient mice on a C57Bl6/J are resistant to hepatic injury following intraperitoneal acetaminophen [52]. Although not the subject of this paper, these authors also firmly show that IL-4 deficient mice develop significantly less protein adducts in their livers. These liver protein adducts could be formed by covalent modification of liver tissues following metabolism of acetaminophen. However, whether or not these protein adducts may serve as neoantigens that stimulate immune response promoting the development of hepatitis following acetaminophen is not completely clear even though these IL-4 deficient mice had significantly lower levels of serum liver enzymes suggesting less toxicity.

5.3 IL-4 in experimental anesthetic drug hapten-induced DILI

Immune-mediated DILI represents a unique condition where immune responses may be induced by drug haptens, native proteins or even previously unrecognized neoantigens formed following the covalent modification of the native protein by the drug hapten [7,9,53]. In our model of experimental immune-mediated DILI, IL-4 has a critical role in initiating CD4⁺ T-cell reactions to key antigens CYP2E1 and the TFA hapten [10]. Interestingly, although the ultimate outcome is an induction of pro-inflammatory responses in this model, IL-4 has separate roles when inducing immune responses to the CYP2E1 autoantigen or the TFA hapten.

Our studies strongly suggest protective roles for IL-10 and possibly IFN- γ in immune-mediated DILI and not IL-4. We have shown that IL-4 promotes the development of immune-mediated anesthetic DILI by suppressing IL-10 or IFN- γ promoted regulatory responses to the CYP2E1 autoantigen during the initiation of this model. Our results also confirm our notion of a direct role for IL-4 in the induction of immune responses to self proteins similar to autoimmune myocarditis in AJ mice or even activation of dendritic cells in EAE. We cannot ignore, however, that while a pro-inflammatory role for IL-4 may support the notion of decreased hepatitis in IL-4 deficient mice, it is also possible that suppression of regulatory immune responses to CYP2E1 in the initiation of this model may promote the development of chronic, but decreased disease in IL-4 deficient mice, as previously suggested in chronic cutaneous leishmaniasis [54] as well as experimental CIA [19]. Even so, this chronic form may have a role either in propagating immune-mediated DILI or even promoting hepatic regeneration or repair following the induction of immune-mediated DILI. Along these lines, our experiments suggest that IL-4 may also suppress IL-6 expression in response to the CYP2E1 autoantigen. This finding further supports a role of IL-4 in the initiation of experimental DILI because it is well known that IL-6 promotes hepatic regeneration and thus protects the liver against various forms of injury such as ischemia, toxins and Fas activation-induced cell death [55,56].

We have also shown that IL-4 promotes the development of immune-mediated DILI by increasing pro-inflammatory (TNF, IL-1 β) and acute phase (IL-6) responses to the TFA hapten. This finding demonstrates that IL-4 induces these cytokines following recognition of TFA-haptenated proteins by activated CD4⁺ T cells and is directly supportive of our notion that IL-4 induces pro-inflammatory responses to the TFA hapten in immune-mediated DILI (Figure 3). Moreover, our findings are directly supported by a previous study in acetaminophen toxicity where IL-4 deficient mice were utilized [52] even though the mice used in this study were C57Bl/6J while our experimental model used BALB/c mice. Taken together, in experimental acetaminophen toxicity and in experimental, anesthetic, immune-mediated DILI, IL-4 increases drug metabolism by inducing mRNA and protein expression

of CYP2E1. In anesthetic DILI, this may produce more reactive haptens and subsequently more neoantigens. These increased neoantigens initiate pro-inflammatory responses in secondary lymphoid organs which culminate in immune-mediated DILI in susceptible mice. What is just as fascinating to us is that, we also show that IL-4 can also demonstrate duality in the expression of IL-6 where IL-4 inhibits IL-6 in response to the CYP2E1 autoantigen but induces IL-6 in response to the TFA hapten, further underscoring the complexity of the pathogenesis of immune-mediated DILI.

6. Conclusion

IL-4 is the complicated cytokine whose role varies between anti- and pro-inflammation in autoimmunity. However, in experimental, immune-mediated liver injury, IL-4 is clearly pro-inflammatory where the target cells can be CD4⁺ T cells or even macrophages. In experimental DILI, whether hepatotoxicity or drug haptens mechanisms are being investigated, IL-4 is clearly pro-inflammatory; however, in response to autoantigens, IL-4 has an immunoregulatory role. Immune-mediated DILI represents a unique condition where hepatitis can be initiated by drug haptens or self proteins, giving IL-4 the opportunity to demonstrate seemingly opposing immune responses depending on whether the stimulating antigen is the hapten or the autoantigen. We have found critical roles for CD4⁺ T-cell reactions to key antigens CYP2E1 and TFA in the initiation of experimental anesthetic DILI by suppressing regulatory cytokine responses to the CYP2E1 autoantigen and by inducing pro-inflammatory cytokine responses to TFA haptenated proteins. Uncovering a pivotal role for IL-4 in our experimental model is especially important in anesthetic DILI because we have previously shown that people with anesthetic DILI develop CYP2E1 autoantibodies of the IgG4 subclass, which would be formed through immune pathways requiring IL-4 signaling [15]. In addition to our findings, we are encouraged by previous reports strongly suggesting a critical role for IL-4 in immune-mediated DILI caused by diclofenac [16]. More importantly, these studies underscore the translational uniqueness of our experimental model because we have been able to first show dual roles for IL-4 in the pathogenesis of autoimmune phenomena in immune-mediated DILI using a single animal model. Ongoing investigations in this arena will further define roles of IL-4, neoantigens and additional immune cells in immune-mediated DILI from halogenated volatile anesthetics or other drugs that have been associated with DILI, such as tienilic acid, dihydralazine, carbamazepine and diclofenac.

7. Expert opinion

We have presented evidence for at least dual roles for IL-4 in the development of experimental, immune-mediated DILI. However, the real question is whether the role of IL-4 in this experimental disease can be translated to patients with immune-mediated DILI. The even larger question is whether key IL-4-driven mechanisms responsible for the pathogenesis of immune-mediated DILI can be translated to other inflammatory diseases of the liver. To answer the former question, we discuss clinical evidence that supports the role of IL-4 in patients with anesthetic DILI. To answer the latter question, we present evidence for common mechanisms in inflammatory liver disorders such as chronic hepatitis C.

Finding significantly elevated CYP2E1 IgG4 autoantibodies in patients with anesthetic DILI has solidified the role for IL-4 in this process. Moreover, it has given clinicians an IgG subclass- specific sera marker that can be used to confirm the diagnosis of anesthetic DILI, because we had previously shown that CYP2E1 IgG antibodies are detectable in the sera of people exposed to halogenated anesthetics in their working environment [14]. Additionally, key mechanisms involving complement activation have been uncovered [15]. Prior to 1992, astute investigators observed low levels of complement components in people with immune-

mediated DILI [57–59]. While the roles for these findings in the pathogenesis of immune-mediated DILI were not clear at that time, a later study has shown that IgG4 autoantibodies can inhibit complement activation and thus prevent clearance of immune complexes [60]. In addition, liver injury in immune-mediated DILI may occur through IgG4-containing immune complexes by mechanisms similar to those suggested in idiopathic membranous nephropathy [61] where IgG4-containing immune complexes escape clearance by the complement system because of their small size. Thus, clarifying roles for IL-4 in anesthetic DILI has increased the diagnostic armamentarium for the clinician by uncovering a more specific marker for active anesthetic immune-mediated DILI as well as confirming a critical role for complement and its measurable components in this process. These findings firmly suggest that autoantibodies of the IgG4 subclass may have a role in immune-mediated DILI from other causes where drug haptens are a prominent feature such as tienilic acid, dihydralazine, carbamazepine and diclofenac.

Uncovering CYP2E1 IgG4 subclass autoantibodies and a significant role for IL-4 in these pathogenic mechanisms could have an additional function in patients with anesthetic DILI. A previous study has clearly shown that sera from people with anesthetic DILI can decrease CYP2E1 enzymatic activity [13], suggesting that these patients may have a diminished ability to metabolize certain drugs. One way to better manage these patients would be to identify the immunogenic epitope of CYP2E1 that may be responsible for this autoantibody. Interestingly, the presence of an IgG4 subclass autoantibody in these people may begin to partially explain this decrease in CYP2E1 activity. It is well known the IgG1 and IgG4 have markedly different structures. IgG4 is known for its compact structure and could potentially explain a pathogenic role for this subclass autoantibody in blocking the active site of the CYP2E1 enzyme. However, presently, we do not have evidence for intracellular penetration of this antibody in order to produce this effect. Even so, recognition of the epitope responsible for this IgG4 subclass antibody could also uncover the critical epitope responsible for T-cell proliferation and initiation of this type of DILI. Hence, once the epitope is identified we can understand its role in the pathogenesis of anesthetic DILI in context of the IgG subclass. We will then be able to map the epitope and its subclass autoantibody onto the crystalline structure of CYP2E1 [62]. Confirmation of this marker by epitope and IgG4 subclass would help to identify patients at risk for developing decreases in CYP2E1 enzymatic activity and may help to prevent the development of toxic reactions to certain drugs such as acetaminophen and halogenated volatile anesthetics that are metabolized by this enzyme. We will then hope that uncovering IL-4-driven these pathogenic mechanisms will have a key role in formulating pharmacological agents that do not trigger this cytokine. We realize that we cannot fully reconcile all of the pro- and anti-inflammatory roles for IL-4 in this review and want to emphasize that IL-4 responses could be affected by the animal models utilized, the disease state of the animal, other medications and possibly the drug doses. The recognition of the roles for these variables in experimental models further establishes the need for clinical studies confirming their significance in human disease states.

It has become increasingly clear to us that the larger question is whether key IL-4-driven mechanisms responsible for the pathogenesis of immune-mediated DILI can be translated to other inflammatory diseases of the liver. Infectious processes have long been associated with the development of autoimmunity, but what may also be occurring is that there are common mechanisms that may initiate and/or propagate chronic hepatic inflammatory conditions. Along these lines, chronic HCV can also induce autoimmune responses. In fact, CYP2E1 autoantibodies have been detected in adult patients with chronic HCV [63]. By demonstrating CYP2E1 autoantibodies in chronic HCV patients without previous ethanol exposure, these astute investigators have shown that HCV infection may induce hepatocellular injury through autoimmune responses to CYP2E1 occurring by the breaking

of self-tolerance against CYP2E1. Interestingly, a recent study has associated chronic HCV with alterations in levels of drug metabolizing enzymes including CYP2E1 [64]. Detecting alterations in CYP2E1 metabolism as well as CYP2E1 autoanti-bodies would suggest that a common CYP2E1 epitope and possibly the IgG4 subclass may be responsible for these phenomena in chronic HCV as well as in anesthetic DILI. Thus, investigations surrounding the dominant CYP2E1 IgG subclass will help to discover if IL-4 driven mechanisms have a pathogenic role in this process. In further support of these lines of investigations, an even more recent study in HCV patients has clearly associated the presence of CYP2E1 autoantibodies with the risk of severe necroinflammation following liver transplantation in adult patients with post transplant HCV [65]. Taken together, these studies in patients with chronic HCV strongly suggest the possibility of common IL-4-driven mechanisms in these patients as well as in patients with anesthetic DILI. Additional research in this area would help to determine if IgG subclass-specific CYP2E1 autoantibodies can identify severe histologic phenotypes of chronic HCV in addition to people with immune-mediated anesthetic DILI. If these studies are subsequently validated in large populations of patients, CYP2E1 subclass-specific autoantibodies may serve as a less invasive marker that can be used to identify all of these patients.

At last, we cannot ignore the growing body of evidence identifying liver disease as a major cause of death and morbidity in people infected with HIV [66]. It is not surprising also that these people are at an increased risk for contracting other infectious diseases such as HCV or even HBV. The presence of HIV in addition to HCV or HBV represents a therapeutic challenge to the clinician because the risk of developing drug-related hepatotoxicity appears to be increased in these patients. Interestingly, IL-18 has been shown to enhance viral replication in HIV as well as promote wasting and possibly dementia [67]. Finding a role for IL-18 may be a crucial link to the role of IL-4 in HIV/AIDS because IL-18 promotes IL-4 expression in CD4⁺ T cells, basophils and mast cells [50]. Presently, it is not known whether CYP2E1 autoantibodies are detectable in HIV patients or in this combined infection group and whether or not their correlation with severity of injury is also relevant in this group. However, demonstrating a role for IL-4 as well as an increased risk for drug hepatotoxicity in HIV with or without HCV or HBV is interesting enough to pose the question.

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Article highlights

- Understanding the role of IL-4 in the pathogenesis of experimental immune-mediated drug-induced liver injury (DILI) offers a pathway to understanding mechanisms of morbidity and mortality in patients with immune-mediated DILI and other inflammatory diseases of the liver.
- IL-4 induces regulatory, suppressive or even pro-inflammatory responses on diverse cell populations. JAK1/STAT6 intracellular signaling is a major pathway for these functions. CD4⁺ T-cell antigen recognition through upregulation of MHCII surface expression is also a critical function for IL-4.
- IL-4 can induce pro- or anti-inflammatory pathways resulting in up or downregulation of immune responses to autoantigens in experimental autoimmune disease models. The ultimate role of IL-4 in the pathogenesis of these disease models may depend on the antigen or adjuvant used to induce the model, the mouse strain used in the model as well as the stage of the disease.
- IL-4 has a pro-inflammatory role in the development of experimental, immune-mediated hepatitis induced by CD4⁺ T cell or granulocytic stimulation from concanavalin A or high dose lipopolysaccharide, respectively.
- IL-4 promotes the development of experimental DILI from carbon tetrachloride, acetaminophen and anesthetic drug hapten-induced DILI by using multiple immunoregulatory mechanisms.
- Hepatitis can be initiated by drug haptens or autoantigens in immune-mediated DILI. Further defining the role of IL-4 in the development of neoantigens, its actions on immune and non-immune cells will clarify the pathogenesis of immune-mediated DILI from halogenated volatile anesthetics, tienilic acid, dihydralazine, carbamazepine and diclofenac.
- Identification of the key autoantigen epitopes responsible for the initiation of immune-mediated DILI is a critical step in reducing the morbidity and mortality of patients with this type of liver injury. Understanding the role of IL-4 in immune-mediated DILI may also assist in understanding the pathogenesis of other chronic inflammatory diseases of the liver such as chronic HCV and HIV in conjunction with HCV or HBV.

This box summarizes key points contained in the article.

Figure 1. IL-4 acts on diverse cell populations

IL-4 induces pro-inflammatory, suppressive and even regulatory responses on immune or non-immune cells. All of these effects may have a role in the pathogenesis of immune-mediated liver injury from drug haptens.

T_H: Helper T cell.

Figure 2. IL-4 JAK1–STAT6 signaling pathway

The IL-4 receptor is formed by the two side chains IL-4Rα as well as the IL-2Rγ chain [28]. IL-4 engagement induces tyrosine phosphorylation of JAK1, which is associated with the IL-4Rα chain, as well as JAK3 phosphorylation, which is associated with the IL-2Rγ chain. STAT6 will bind to the phosphorylated receptor allowing its phosphorylation by JAK1. The phosphorylated STAT6 dislodges from the receptor and forms homodimers. STAT6 homodimers translocate to the cell nucleus where the homodimers are disrupted through enzymatic action by STAT6 proteases. STAT6 then binds to DNA and promotes transcription of MHC II, IL-4 and IL-4Rα.

Figure 3. Proposed mechanism of immune-mediated DILI

In our model of experimental, murine, immune-mediated, anesthetic DILI, BALB/c mice develop an IL-4 promoted hypersensitive response to the TFA hapten and S100 liver proteins resulting in splenocyte TNF- α , IL-1 β and IL-6 production. IL-4-dependent splenocyte proliferation is also demonstrated in response to human CYP2E1 and TFA through suppression of IL-10 and possibly IFN- γ regulatory responses. Immune responses to CYP2E1, S100 and TFA also activate mast cells that recruit neutrophils to the liver. Directly or indirectly through IL-10, a predominantly IL-4-driven T_H2-type response leads to S100 and CYP2E1 autoantibody production as well TFA hapten antibodies. Reproduced with permission from Eur J Immunol. DILI: Drug-induced liver injury; TFA: Trifluoroacetyl chloride; T_H: Helper T cell.

Table 1

Roles for IL-4 in classical experimental autoimmune disease models.

Anti-inflammatory	Pro-inflammatory
Experimental diabetes	Experimental autoimmune encephalitis (?)
Experimental autoimmune encephalitis	Collagen induced-arthritis
Collagen-induced arthritis Myocarditis	Myocarditis

Table 2

Pro-inflammatory roles for IL-4 in experimental immune-mediated hepatitis.

Model	Targeted cells
Concanavalin	CD4 ⁺ T cells
High dose lipopolysaccharide	Macrophages