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Invited review

Animal models of autoimmune hepatitis☆

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ABSTRACT

Many animal models for autoimmune hepatitis (AIH) have been described in the past. Most models had to deal with the relative immunosuppressive environment of the liver. Therefore, some models used a combination of several triggering factors often on a susceptible background to generate an aggressive immune response that targets the liver. In addition, in order to be able to track the immune response the models used specific model autoantigens as targets that are either not present or have not been identified as a natural autoantigen in AIH patients. Thereby the feasibility of such models is somewhat questionable. Although many historic approaches included challenges of experimental animals with liver homogenates it was only in the last decade that natural occurring liver autoantigens have been used in animal models. This article reflects on the requirements for breaking liver tolerance and on how an ideal experimental model for AIH would look like. In addition, it discusses historic as well as recent animal models in the context of feasibility of induction, similarity of the clinical outcome to human AIH, and gain of knowledge for possible future therapies.

1. Introduction

Many models for autoimmune liver diseases have been described. In general, such models had two major goals. First, to understand more about liver immunology including its immunosuppressive features. Second, to cause autoimmune-mediated liver damage that closely reflects the clinical features of human autoimmune liver diseases, such autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), or primary sclerosing cholangitis (PSC), in order to evaluate novel therapeutic strategies for human disease.

To achieve the first goal, it is important to use components that have been already characterized and allow for precise tracking of the immune response. Thus, many research groups used well-characterized model antigens that are transgenically expressed as targets in the liver. In addition, a transfer of a defined T cell population isolated from TcR-transgenic mice facilitates the tracking and characterization of autoaggressive T cells after adoptive transfer. The problem with such transgenic approaches using model antigens is its feasibility as they are often not related to the human disease. Yet, to be able to investigate basic mechanisms involved in liver cell destruction and if present hepatic fibrosis, it is of course important to be successful in breaking immune tolerance in the liver. Thus, many of these transgenic models revealed novel mechanisms of immune activation as well as immune regulation. To be sure to elicit a strong immune response in the liver, several aspects would have to be fulfilled. First, a certain degree of susceptibility, which may include the ideal MHC haplotype fit for presenting critical epitopes of an autoantigen or a defect in immune regulation as occurring in spontaneous disease models like the non-obese diabetic (NOD) mouse that is predominantly used to study type 1 diabetes (T1D) [1]. Second, a target autoantigen whose expression is transgenically controlled and restricted to the liver. Third, the presence of a strong T cell response with specificity to the target autoantigen. This can be accomplished by either directly using TcR-transgenic mice as model system or by adoptively transferring T cells isolated from TcR-transgenic mice. Forth, a strong inflammation of the liver that provides critical inflammatory factors that ensure activation and proliferation of autoantigen-specific T cells.

To achieve the second goal, namely the generation of a model that is as close as possible to the human disease in order to have a tool for evaluating possible therapies, it is important that the path of disease induction is feasible and that the clinical phenotype, including serology and histology is similar to the one found in patients. A realistic scenario for the development of AIH in patients would be one or a series of triggering event(s) that would induce liver autoimmunity in susceptible individuals. It is the current opinion that most autoimmune diseases develop due to a combination of genetic and environmental factors [2]. Molecular mimicry has been proposed as a concept by which the impact of environmental factors might be potentiated and autoimmune processes accelerated. Thus, the molecular mimicry hypothesis has been used as a basis for experimental animal models for many autoimmune

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2. Autoimmune hepatitis: clinical features, autoantibodies, and treatment

AIH is a serious autoimmune liver disease that, in contrast to PSC and PBC, primarily affects the liver parenchyma, rather than the bile ducts. As a consequence hepatocytes are progressively destroyed resulting in subsequent chronic fibrosis [3–7]. According to the World Health Organisation AIH has an annual incidence of approximately 2 in 100,000 individuals and a prevalence 15 cases per 100,000 persons worldwide (http://www.who.int/ipcs/publications/ehc/ehc236.pdf). Alone in the USA an estimated 100,000 to 200,000 persons are affected by AIH [8,9]. AIH has a female predominance (sex ratio, 3.6:1), occurs in children and adults of all ages and affects several ethnic groups [10]. The clinical spectrum of AIH is rather variable and ranges from asymptomatic presentation to severe forms of hepatitis that may be almost indistinguishable from acute viral hepatitis or fulminant hepatic failure [5,11,12]. Due to such varying clinical signs and partial overlaps with symptoms of PBC and PSC a conclusive diagnosis of AIH remains challenging [5,6,11–13]. Thus, in 1999 the International AIH Group (IAIHG) agreed on a diagnostic scoring system to aid in the diagnosis of AIH [14]. However, subsequent experience in clinical practice revealed the complexity of this initial scoring system. Thus, in 2008 a simplified scoring system has been postulated that consists of only four parameters, including increased immunoglobulin G concentration (hypergammaglobulinemia), absence of viral markers, typical histological features compatible with AIH, and presence of specific autoantibodies [15]. Although serum aminotransferases are frequently elevated in AIH and are in clinical routine the first indicators of liver disease, they have not been considered with a score in the simplified scoring system. The histological hallmark of AIH is the presence of an interface hepatitis / piecemeal necrosis, which affects patches of hepatocytes. Often such regions are characterized by plasmacytosis (infiltrating plasma cells), hepatocyte rosetting and emperipolesis [5,7,11].

Besides histologic evaluation of liver biopsies, one of the main diagnostic criteria of AIH is the presence of specific autoantibodies to particular liver autoantigens that also allow for a classification of AIH into subtypes [16,17]. In general, the presence of anti-nuclear (ANA) and/or anti-smooth muscle (SMA) autoantibodies characterizes AIH type 1 (AIH-1), whereas type 1 liver/kidney microsomal autoantibodies (LKM-1) are the hallmark of AIH type 2 (AIH-2) [5,10,11,18]. In general, patients with AIH-1 and AIH-2 differ in clinical presentation and course of disease [19]. However, a recent study with 26 and 52 patients classified as suffering from AIH-1 and AIH-2, respectively, demonstrated that with exception of differences in the presence of individual autoantibodies AIH-1 and AIH-2 patients share a similar clinical phenotype [20]. Since some patients even displayed a change in the autoantibody profile from one subtype to another over time, it had been suggested that in some cases AIH-2 might constitute an early form of AIH appearing in younger patients who later may convert to AIH-1 [20]. Historically, even a third type of AIH (AIH-3) has been postulated to classify patients carrying autoantibodies directed against soluble liver antigen (SLA) and liver and pancreas antigen (LP) (anti-SLA/LP antibodies). However, the subtyping into AIH-3 is considered obsolete since anti-SLA/LP antibodies are often present in context with other autoantibodies pointing towards AIH-1 and the course of disease is similar to AIH-1 [21]. The group of ANA recognize several different components present in the nucleus, including DNA, centromers, histones, sn-RNPs, and cyclin A [22,23]. Since ANA are also found in patients with PBC, systemic sclerosis (SSc), drug-induced hepatitis, chronic hepatitis B or C, and non-alcoholic fatty liver disease (NAFLD), an assessment of the nuclear staining pattern is essential for diagnosis and the mere presence of ANA may be compatible with, but not a bona fide diagnostic marker for AIH-1 [24]. SMA are directed against filamentous actin (F-actin) and represent valid diagnostic antibodies for AIH-1 only if evaluated carefully, since just like ANA, SMA have been found in the sera of patients with other liver diseases with an autoimmune or viral background. However, SMA titres are usually higher in AIH-1. Detailed information on ANA and SMA staining patterns as diagnostic tools is available in two recent review articles [16,24]. The main target for anti-LKM-1 antibodies found in AIH-2 patients has been identified as the 2D6 isoform of the cytochrome P450 enzyme family (CYP2D6) [25,26]. Besides CYP2D6, ERp57 and carboxylesterase 1 (CES1) have been identified as additional LKM-1 target structures present in the microsomal compartment [27]. Anti-LKM-1 antibodies are considered diagnostic, as long as patients are HCV negative, since reactivity to CYP2D6 has also been found in patients with chronic hepatitis C [28–30]. Besides ANA, SMA, LKM-1, and anti-SLA antibodies, patients may also carry additional autoantibodies, such as peripheral anti-nuclear neutrophil antibodies (pANNA) (also termed “atypical” peripheral anti-neutrophil cytoplasmic antibodies (pANCA)), anti-liver and pancreas antigen (LP) antibodies, liver cytosol type 1 antibodies (LC-1), type 2 or type 3 liver/kidney microsomal antibodies (LKM-2 and LKM-3, respectively), anti-liver-specific membrane lipoprotein (LSP) antibodies, and anti-liver membrane antibodies (LMA) [5,31–36]. For a detailed review on AIH autoantibodies, their target structures and their diagnostic value, I would like to refer to a selection of recent review articles [16,17,24].

The recommendation of the European Association for the Study of the Liver (EASL) for the standard therapy of AIH is a glucocorticoid treatment with prednisone or prednisolone alone or in combination with azathioprine [37]. Alternative treatments include the next generation glucocorticoid budesonide [38] as well as the calcineurin inhibitors cyclosporine A and tacrolimus [12,39]. It has been reported that a combination therapy with budesonide and azathioprine resulted in fewer side effects than the conventional prednisone/azathioprine therapy in AIH patients without cirrhosis [38]. However, the EASL clinical guidelines do not recommend budesonide for patients with cirrhosis or peri-hepatic shunting, since the lack of efficient first-pass hepatic clearing of budesonide might result in undesired side effects [37]. A further alternative is the cytostatic immunosuppressant drug mycophenolate mofetil (MMF), which interferes with the purine biosynthesis and has been demonstrated to be safe and effective as first-line or rescue therapy [40]. However, the EASL clinical practice guidelines suggest using MMF mainly as a second-line therapy in cases of azathioprine-intolerance [37]. Unfortunately, long-term standard therapies with glucocorticoids and cytostatic drugs carry the risk of significant steroid-specific and azathioprine-related side effects. This is problematic since standard treatment for only a short period of time is not very effective. Adults rarely achieve resolution in less than 12 months and withdrawal of therapy after only two years of treatment results in relapses in 85% of cases [10].

3. Autoimmune hepatitis: susceptibility and possible environmental triggers

Just like in virtually all other autoimmune diseases the HLA haplotype is the predominant risk factor for AIH. Both AIH-1 and AIH-2 are associated with HLA-B8 (MHC class I) and HLA-DR3 [DRB1*03:01] (MHC class II). Further associations are with HLA-DR4 [DRB1*04:01] (MHC class II) for AIH-1 as well as HLA-DR7 [DRB1*07:01)] and HLA-DQ2 [DQB1*02:01] (both MHC class I) for AIH-2 [41–47]. Recently DRB1*03:01 and DRB1*04:01 have been confirmed as the primary and secondary susceptibility loci for AIH-1 in Dutch and German cohorts by genome wide association study (GWAS) [48]. Distinctive susceptibility variants have been reported for different ethnic groups (see [47] for a more detailed listing of HLA associations) and the HLA-haplotype seems to also influence the course of the disease in that presence of the HLA-B8 allele is associated with a more severe inflammation. Patients carrying an HLA-B8 allele are also more likely to suffer relapses after treatment. Similarly, patients with an HLA-DR3 allele display a lower

frequency of remission and a higher probability for relapses [49]. In contrast, presence of an HLA-DR4 allele seems to have a somewhat protective effect, since HLA-DR4-positive patients have a higher rate of complete remissions and a lower frequency of cirrhosis [50].

Several environmental factors that might induce and/or accelerate a detrimental activation of an aggressive immune response in the liver have been suggested in the past. Such triggers include pathogen infections, drugs, alcohol, and obesity. It has been reported that about 50% (26 out of 54) of patients with non-alcoholic steatohepatitis (NASH) also carry ANA or AMA and display besides features of NASH, also histological signs of AIH or PBC, respectively [51]. However, the influence of obesity on the development of autoimmune liver disease is a matter of debate. On the one hand, a study with patients with non-alcoholic fatty liver disease (NAFLD) demonstrates that a substantial fraction of patients (23% of 225 patients) carried ANA or SMA. The majority (88%) of those ANA or SMA-positive NAFLD patients fulfilled the diagnostic criteria for AIH [52]. On the other hand, another study demonstrated the presence of ANA in 33% of 212 NAFLD patients, but only one has been also classified as definite AIH after liver biopsy [53]. To date there is no firm proof that NAFLD or NASH indeed play a role in the immunopathogenesis of human AIH, a situation that is quite similar to alcoholic liver disease and its influence on autoimmunity [54]. However, it has been shown in a mouse model for AIH (the CYP2D6 model, see section below) that pre-existing NAFLD exacerbates AIH characterized by an increased frequency of liver autoantigen-specific T cells and a higher degree of cellular infiltration and liver fibrosis [55]. Many drugs have been identified to cause acute and/or chronic damage to the liver. Whereas some drugs are toxins that cause direct cellular damage, some drugs are enzymatically converted to reactive metabolites that form protein adducts. Such modified self-proteins might cause autoimmune-mediated hepatitis with similar manifestations as AIH. However, due to their known etiology, drug-induced hepatitis, such as Halothane hepatitis [56], are classified separate from AIH [57]. The question if such drug-induced hepatitis may also break tolerance to AIH-related liver autoantigens and thereby induce or accelerate AIH has yet to be answered. In PBC however, it has been suggested that the cosmetic and food additive 2-octynoic acid (2-OA) can trigger an immune response against the main PBC autoantigen, namely the E2-subunit of the pyruvate dehydrogenase complex (PDH-E2) [58]. Patients with PBC carry autoantibodies directed against the PDH-E2 that cross-react with 2-OA [58] and immunization with a 2-OA conjugated carrier protein induces a PBC-like disease in mice [59]. The structural similarity between 2-OA and the lipoic acid moiety in PDH-E2 is a typical example of a phenomenon called ‘molecular mimicry’, in which antibodies and/or T cells cross-react to trigger and target.

Most often molecular mimicry has been demonstrated for structures present on pathogens and hosts [60,61]. Predominantly virus infections have been implicated in the initiation and/or propagation of AIH [62]. The most compelling association has been found for HCV, since LKM-1 antibodies have been also found in HCV-infected patients and antibodies binding to the HCV proteins NS3 and NS5a cross-react to a specific conformational epitope on the major AIH-2 autoantigen CYP2D6 [63]. Further, LKM-1 antibodies specific for another CYP2D6 epitope cross-react with epitopes on proteins of HCV as well as human cytomegalovirus (HHV-5) [64]. Further sequence homologies have been found between CYP2D6 and a variety of human pathogens including herpes simplex virus 1 (HSV-1) [65], Legionella pneumophila, Influenza A virus (H1N1), and Kaposi's sarcoma associated herpes virus (HHV-8) [66]. However, similar to many other autoimmune diseases with associations with pathogen infections, there is yet no definite proof for pathogens to be directly involved in the initiation and/or acceleration of AIH. For more information about a possible role of pathogen infection on AIH, I would like to refer to a more detailed review article [2].

4. Relative immune tolerance in the liver

Compared to other autoimmune diseases, such as autoimmune thyroiditis, multiple sclerosis (MS), or type 1 diabetes (T1D), the frequency of autoimmune diseases affecting the liver is rather low. Considering that the liver is the main organ responsible for detoxification and drug-metabolism and is therefore prone to cellular damage, such a low frequency of autoimmune liver diseases might come as a surprise. First, cellular damage may lead to local inflammation and activation of resident immune competent cells, such as Kupffer cells (KC) or liver sinusoidal endothelial cells (LSEC) and subsequently the attraction of infiltrating lymphocytes that may cause specific or unspecific damage to the liver parenchyma. Second, the metabolism of some drugs and xenobiotics results in the formation of reactive metabolites with the potential to covalently attach to macromolecules, such as proteins, lipids, or even DNA [67,68]. Such modified self-components may act as neo-antigens causing an aggressive immune response, as extensively demonstrated for the anesthetic agent halothane that can cause severe halothane hepatitis in susceptible individuals [69]. Third, the liver is target of many pathogens, including Hepatitis-, Coxsackie- and Herpes simplex viruses. Such infections directly damage hepatocytes and other liver-resident cells and cause a strong local inflammation. Thus, pathogen infections of the liver might act as triggering factor for subsequent autoimmune reactivity [62].

As protection from autoimmunity many mechanisms have evolved to regulate the immune balance in the liver. With the help of several animal models such protective mechanisms, some of which are unique to the liver, have been identified. For example, LSEC cross-present antigenic liver peptides resulting in T cell inactivation [70,71] and induction of antigen-specific regulatory T cells [72] and thereby maintain hepatic tolerance to liver autoantigens. Further, hepatic stellate cells (HSC) have been shown to induce T cell apoptosis [73]. The tolerogenic environment of the liver has been impressively demonstrated by Lüth et al. [74]. They found that an ectopic expression of myelin basic protein (MBP, a major autoantigen in MS) in the liver, but not in the skin, resulted in the protection from neuroinflammatory disease in a mouse model. MBP-expression in the liver caused the generation of MBP-specific regulatory T cells, which inhibited the proliferation of MBP-specific aggressive T cells. These regulatory T cells were sufficient to protect from MS-like disease, since adoptive transfer into mice not expressing MBP in the liver also protected from disease [74]. In summary, the liver microenvironment seems to be a milieu that regulates peripheral tolerance and prevents excessive damage of the liver by autoimmune dysregulations. Thus, in order to generate a model for any autoimmune-mediated liver disease one has to overcome such tolerogenic mechanisms.

5. Animal models

As mentioned beforehand an ideal experimental model system would be as close as possible to the human disease. However, mice that are predominantly used to model human autoimmune diseases often do not share the same target autoantigens and in particular due to differences in the MHC class I and II molecules the specificity of the T cell response is different in mouse and men. Table 1 provides a summary of the most important features of human AIH and a listing of how these features have been reproduced in the collectivity of animal models generated. The individual models are described in detail in the following paragraphs and in Table 2. Naturally, none of the listed models perfectly reflects the human situation. However, as discussed below some models are better suited to investigate basic mechanisms of immune activation and/or regulation and some are more likely to help assessing novel therapies. In addition, it is important to realize whether a given model reproduces an acute or a chronic form of hepatic destruction.

Naturally almost all animal models have been heavily influenced by

Table 1
Comparison of human AIH and experimental AIH.

Feature	Human AIH	AIH models
Etiology	- Unknown - Likely a combination of genetic and environmental factors	- Spontaneous models with transgenic modifications - Inducible models requiring a trigger (liver tropic pathogen and/or transfer of antigen-specific T cells)
General outcome	- Chronic disease - Progressive destruction - Liver fibrosis	- Acute hepatitis models (ConA hepatitis) - Many transient hepatitis models - Only few models with chronic hepatitis (CYP2D6 and FTCD models)
Serology	- Elevated aminotransferase levels - Hypergammglobulinaemia	- Transient or persistent elevation of aminotransferase levels in almost all models - Hypergammaglobulinaemia reported for some models (FAH model)
Antibodies	- Several autoantibodies - Some with clearly defined and characterized targets (i.e. LKM-1 → CYP2D6) - Some rather diffuse with many targets (i.e. ANA)	- Presence of autoantibodies are often reported - Extensive characterization of CYP2D6 antibodies (incl. Epitope spreading) - Generation of ANA, anti-SMA, anti-SLA
T cells	- Several subtypes of antigen-specific T cells (Type 1, 2, and 3 effector T cells as well as regulatory T cells)	- Detailed characterization of T cell activation or anergy in TcR transgenic models (i.e. Alb-HA/CL4 model) - Generation of antigen-specific T cells (CYP2D6 and FTCD models) - Type 1 domination after viral trigger (CYP2D6 model)
Histology	- Interface hepatitis with massive cellular infiltration - Piecemeal necrosis - Plasmacytosis - Bridging fibrosis	- Moderate to strong interface-like hepatitis with cellular infiltration in almost all models - Only few models with necrosis (Tyro3/Axl/Mer KO model) - Plasma cells are often detected, but rarely quantified - Some models display various forms of fibrosis (AAV-IL-12, CYP2D6, FTCD models)

observations made in AIH patients. Fig. 1 provided a rough summary of the most important players in the immunopathogenesis of human AIH. Since there is no firm proof for one or more environmental factors to be involved in the initiation and/or propagation of the disease, only descriptive observations at or after diagnosis of the disease are being considered. There are several important issues: First, several forms of autoantibodies against antigenic targets in the liver are generated and some of which are used as diagnostic tools. However, a direct pathogenicity of such antibodies has not been demonstrated to date. Second, assessment of serum inflammatory factors has revealed that many soluble pro-inflammatory factors, such as chemokine and cytokines, as well as pro-fibrotic are elevated in AIH-patients. Third, a disturbed balance of the immune system has been detected with elevated levels of type 1 (Th1/Tc1) and type 3 (Th17/Tc17) T cell subtypes. Critical inflammatory factors may certainly constitute possible targets for immune intervention. Thus, to be able to evaluate therapies, such as a specific blockade of a critical cytokine, the corresponding inflammatory factor or cell population should also play a crucial role in the pathogenesis of AIH-like disease in the model system.

6. Animal models: pre-transgenic area

The first animal models have been generated in the early 1970s during a period of time when more and more of the basic mechanisms of the immune system have been identified and it has become clear that AIH has an immune mediated background. Meyer zum Buschenfelde and colleagues used two crude fractions of human liver homogenates, which contained one of the two liver-specific antigens LP-1 or LP-2 known at the time. They injected these fractions together with various adjuvants into rabbits and found that LP-1, that has been described as a water insoluble macromolecular low-density lipoprotein, was efficient in inducing “experimental immune hepatitis” [75]. In the meantime, LP-1 is known as LSP and has been identified as the asialoglycoprotein receptor (ASGPR), which is highly expressed at the surface of hepatocytes [31]. Anti-LSP antibodies are present in up to 88% of AIH-patients [31], and may be used as a general marker compatible with AIH, but not as a diagnostic tool, since such antibodies have also been detected in patients with chronic hepatitis B and C, alcoholic liver disease, and PBC [49]. Although, the approach was simple and the real identity of both the triggering antigen as well as the target liver autoantigen has not been known at that time, Meyer zum Buschenfelde et al. succeeded in inducing a state of autoimmune liver damage characterized by a diffuse portal and periportal infiltration with lymphoid cells and piecemeal necrosis [75]. Interestingly, the rabbits generated antibodies to LP-1 and LP-2, but these antibodies were not sufficient to induce AIH-like chronic hepatitis in transfer experiments, indicating the additional requirement of pathogenic T cells and/or a strong local inflammation in the liver [75]. A similar approach has been made by Kuriki et al. in the 1980s, who injected syngeneic liver homogenate or liver-specific lipoproteins with the polysaccharide of *Krebsiella pneumoniae* 03:K1 as an adjuvant into SMA mice, resulting in generating liver-specific lipoprotein antibodies and portal infiltrations of mononuclear cells in the liver [76]. In analogy to the then widely used experimental autoimmune encephalomyelitis (EAE) model for multiple sclerosis (MS), they termed their model experimental autoimmune hepatitis (EAH). Importantly, they were able to demonstrate the presence of pathogenic immune cells, since transfer of splenocytes from such EAH-mice into naïve recipients induced similar features as detected in the donor mice with established EAH within 14 days after the transfer. These features included portal infiltrations with mononuclear cells as well as necrosis of liver parenchymal cells [76]. Further, Lohse et al. generated an EAH model by injecting C57BL/6 mice with a crude 100,000 g supernatant of syngeneic liver homogenate (S-100) emulsified in complete Freund's adjuvant [77]. The important novel finding was that besides a transient liver damage (perivascular infiltrates and hepatocyte necrosis) the generation of S-100 protein-specific T-cells has been demonstrated [77]. A few years earlier, Watanabe et al. used a very similar approach by injecting inbred A/J mice subcutaneously eight times with the supernatant of liver homogenate centrifuged at 105,000*g* [78]. Although they did not use the term S-100, their liver extract must have been very similar to the one used by Lohse et al. They showed EAH characterized by histological features of hepatitis as well as the production of anti-LSP antibodies and a strongly pronounced delayed-type hypersensitivity to LSP after neonatal thymectomy. This finding suggests a possible presence of an immunosuppressive T cell component in such EAH mice, since neonatal thymectomy prevents the proper development of the CD4 + CD25 + population of regulatory T cells [79]. Since then variations of the S-100 model have been often used by several research groups worldwide. For example, it has been shown that vaccination with irradiated activated T cells isolated from the liver of EAH, but not naïve mice, evokes an anti-idiotypic regulatory response, directed against epitopes of the Vβ-chains of T cells used for vaccination, and

Table 2
Animal models for human AIH.

Model	Target/transgene	Trigger	Additional requirement	Form of hepatitis	Implementation	Reference
Pre-transgenic area						
Rabbit/mouse	Liver autoantigens	Allogeneic or xenogenic liver proteins		Prolonged flares	Basic mechanisms of AIH (infiltration)	[75]
Experimental autoimm. hepatitis (SMA mice)	Liver autoantigens	Syngeneic liver homogenate Liver-specific lipoproteins	*Krebsiella pneumoniae* polysaccharide	Portal infiltrations Autoantibodies	Basic mechanisms of AIH (antibodies)	[76]
Experimental autoimm. hepatitis (C57Bl/6 mice)	Liver autoantigens	S-100 liver homogenate	Freund's adjuvant (cFA)	Transient with necrosis T cells	Basic mechanisms of AIH (T cells)	[77]
Experimental autoimm. hepatitis (A/J mice)	Liver autoantigens	S-100 liver homogenate	Thymectomy	Autoantibodies	Basic mechanisms of AIH antibodies)	[78,79]
ConA hepatitis (mouse)	T cells	Concanavalin A		Severe, acute liver injury	Evaluation of novel therapies affecting acute inflammation	[83–89]
Transgenic models developing spontaneous AIH-like disease						
NTxPD-1 −/− mice	PD-1 −/− Regulatory T cells	Spontaneous, transgenic	Thymectomy	Fatal hepatitis ANA	Role of T cell regulation	[91–93]
Tyro3/Axl/Mer KO mice	Tyrosine kinase −/− TLR signaling	Spontaneous, transgenic		Elevated aminotransferases Piecemeal necrosis ANA, anti-SMA antibodies	Role of innate immune response	[94]
APS-1 mice	Aire −/−	Spontaneous, transgenic	Severity dependent on mouse strain	Elevated aminotransferases Periportal infiltrates Autoantibodies	Role of central tolerance	[97]
Traf6ΔTEC mice	TRAF6 −/− in thymic epithelial cells	Spontaneous, transgenic		Interface hepatitis Cellular infiltrates ANA, anti-SLA antibodies	Role of central tolerance	[98]
Transgenic models using defined targets and triggers to induce AIH-like disease						
Alb-HBsAg mouse	HBsAg	HBsAg & Transfer of HBsAg-specific T cells		Transient hepatitis Hypersensitivity reaction	Role of liver antigen- specific T cells	[101,102]
CRP-K^b mouse	H-$2K^b$ (MHC I)	Transfer of TCR tg H-$2K^b$-specific T cells		Tolerant	Role of peripheral T cell tolerance	[105]
CRP-K^b / Alb-K^b mouse	H-$2K^b$ (MHC I)	Transfer of TCR tg H-$2K^b$-specific T cells	*L. monocytogenes* IL-2 administration CpG-ODN admin.	Histologic hepatitis	Role of local infection in tolerance	[106,107]
Met-K^b mouse	H-$2K^b$ (MHC I)	Engraftment of TCR tg H-$2K^b$-specific bone marrow	Thymectomy	Transient hepatic infiltrates	Role of central & peripheral tolerance	[108–110]
TF-OVA / ASBT-OVA mouse	Ovalbumin (OVA)	Transfer of TCR tg OVA-specific T cells	liver injury	Acute but transient	Role of peripheral T cell tolerance	[111]
Alb-LCMV-GP33 mouse	LCMV Glycoprotein epitope (GP33)	Transfer of TCR tg GP33-specific T cells	LCMV infection	Transient hepatitis	Role of local infection in tolerance	[112]
Alb-HA/CL4 mouse	Hemagglutinin (Influenza virus)	Spontaneous, TCR tg HA-specific T cells		Persistent hepatitis Anergic T cells High numbers of Tregs	Role of peripheral T cell tolerance & regulation (Tregs)	[114]
Models requiring cytokine triggers to induce AIH-like disease						
TTR-LCMV-NP mouse	LCMV Nucleoprotein (NP)	DNA-vaccination (NP & IL12)		Elevated aminotransferases Minor infiltrates	Role of IL-12	[116]
Wildtype mice (C57BL/6, 129/Sv Balb/c)	CYP2D6 + FTCD	DNA-vaccination (CTLA-4 & CYP2D6 & FTCD & IL-12)		Minor hepatitis Autoantibodies Specific T cells	Role of CTLA-4 Evaluation of novel therapies affecting T / B cells	[117]
C57BL/6 mice	Liver autoantigens	Vaccination with HCC cell loaded DCs	IL-12 administration	Liver inflammation S-100 reactive splenocytes	Role of antigen presentation	[118]
C57BL/6 mice	Liver autoantigens	Infection with AAV-IL-12		Elevated aminotransferases Cellular infiltrates Hepatic fibrosis ANA, anti-SMA antibodies	Evaluation of novel therapies affecting infiltrates, B cells & fibrosis	[119]
Models using natural occurring autoantigens						
CYP2D6 model (FVB, C57BL/6 mice)	Mouse Cyp homologues to human CYP2D6	Adenovirus hCYP2D6 infection		Chronic hepatitis Autoantibodies Specific T cells Persistent fibrosis	Evaluation of novel therapies affecting infiltrations, T / B cells & fibrosis	[120–123]
FTCD model (NOD mouse)	FTCD	Adenovirus FTCD infection	Severity dependent on mouse strain	Chronic hepatitis Fibrosis	Evaluation of novel therapies affecting infiltrations & fibrosis	[124]
FAH model (C57BL/6 mice)	FAH	MHV infection	Peptide adjuvant PADRE	Elevated AST/ALT Hypergammaglobulinemia Liver infiltrations Autoantibodies	Evaluation of novel therapies affecting B cells	[125]

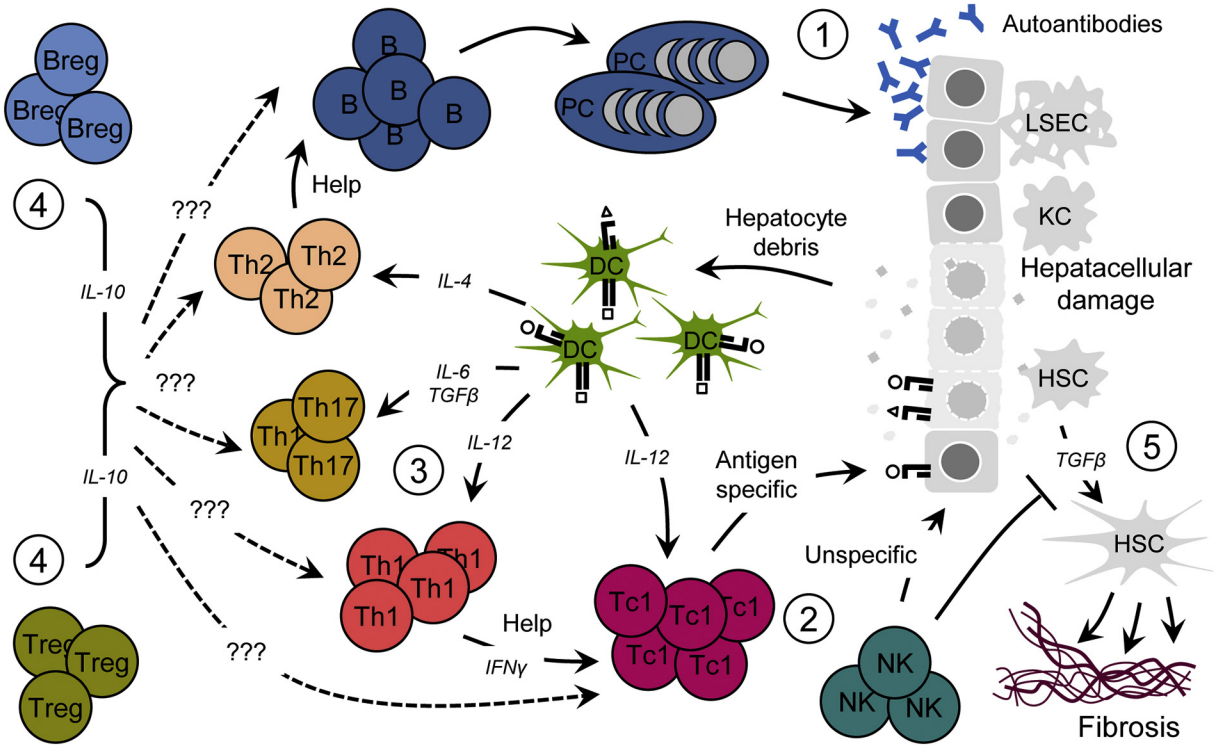

Fig. 1. Major players in the immunopathogenesis of human AIH - (1) Autoantibodies against liver antigens are the hallmark of both types of AIH and are used as diagnostic markers. However, the is currently no hard evidence that such autoantibodies are pathogenic. – (2) Hepatocytes are killed by in unspecific or antigen-specific manner by NK cells or T cells with cytotoxic activity (Tc1, type 1 CD8 T cells), respectively. – (3) Professional antigen presenting cells, such as dendritic cells (DC), but also the liver resident Kupffer cells and LSECs with cross-presentation activity, further propagate T cells subtypes. Depending on the local inflammatory milieu the immune balance is shifted towards a type 1 (Th1/Tc1), type 2 (Th2/Tc2), or type 3 (Th17/Tc17) phenotype. – (4) Under inflammatory conditions an additional decrease in immune cells with a regulatory phenotype, such as regulatory T cells (Treg) or B cells (Breg) expressing IL-10 might contribute to the chronic nature of AIH. However, the actual impact of such regulatory cells, or the insufficient number thereof, on the immumnopathogenesis of AIH is not yet clear. – (5). HSC are activated in the inflammatory milieu of AIH and are mainly responsible for the extensive generation of extracellular matrix proteins, such as collagen I, that builds up the fibrotic tissue.

protects recipient mice from S-100 induced EAH [80]. More recently, the S-100-induced EAH model was used to demonstrate that the mitogen-activated protein kinase (MAPK) p38 and the transcription factor nuclear factor kappa B (NF-κB) play an important role in the immunopathogenesis of AIH [81]. Blockade of p38, which normally reaches its peak of expression between days 14 and 21 after S-100 administration, resulted in a decreased NF-κB activation and subsequently a reduced expression of pro-inflammatory cytokines, such as IFNγ, TNFα, IL-1β, and IL-12. Due to such a reduced pro-inflammatory milieu in the liver, cellular infiltrates were diminished, and serum aminotransferase levels decreased [81]. The S-100 model has also been used to investigate the suppressive role of regulatory B cells expressing CD11b [82]. Thereby, Liu et al. showed that IL-10-dependent expression of CD11b on B cells rendered a regulatory state in such B cells. CD11b B cells mediated their suppressive/regulatory function through impairment of TcR signal transduction and promotion of TcR down-regulation, resulting in CD4 T cell suppression and amelioration of S-100-induced EAH [82]. In summary, these models originated in the "pre-transgenic" area when many of the target autoantigens have not yet been identified and they were based on the injection of liver extracts with largely unknown compositions. Today, it is known that such a microsomal subcellular fraction, such as the S-100 extract, contains several liver autoantigens, such as CYP2D6 [26] and SLA/LP [33]. Nevertheless, these crude models gave valuable information on the presence of effector cells and regulatory phenomenon in liver-specific immune reactions. The advantage of the S-100 model lays in its simplicity that does not require transgenic animals or complicated ways of disease induction. However, these early models rarely induced a chronic relapsing AIH with similarities to the human disease and the lack of knowledge about the target antigens prevented the quantification and tracking of liver autoantigen-specific T cell responses.

Systemic application of Concanavalin A (ConA) is another method for inducing liver injury in mice that is frequently used since the early 1990s and leads to antigen non-specific T cell activation and severe acute hepatitis [83]. The Jack bean lectin ConA causes a cytokine storm with high levels of IFN-γ and TNF-α that is amplified by an interaction between lymphocytes and macrophages and subsequently ensues inflammatory lesions, hepatocyte apoptosis, and fulminant hepatic failure [84–86]. Interestingly, the T cell mitogenic power of ConA is strong enough to effectively arm the T cell compartment to successfully oppose hepatoma in a murine model [87]. The pathogenesis of ConA hepatitis has been elucidated in great detail. For example, using CD1d-deficient mice that are lacking NKT cells, it has been demonstrated that NKT cells are essential for ConA-induced liver injury [88]. However, although the ConA-hepatitis model is a reliable model to investigate aspects of immune system-mediated liver damage, it represents more of a model for acute liver injury precipitated by a liver autoantigen-independent cytokine storm rather than a model of chronic AIH. Anyhow, it has been also used to evaluate possible therapies for human AIH. Very recently for example, Park et al. investigated the anti-inflammatory effect of the traditional Korean herbal medicine Yongdamsagan-tang (YST), which is essentially a mixture of several root extracts [89]. They found that YST reduced IL-6 production and blocked the expression of several factors important for hepatic fibrosis, such as TGF-β1, collagen I, and α-smooth muscle actin (α-SMA) and that treatment of mice with YST before administration of ConA protected from hepatic damage [89]. It has to be noted here that other herbal extracts, such as a medicinal herbs mixture containing *Scutellaria baicalensis* (Sb) and *Bupleurum chinese* (Bc) that

have been sporadically reported to be associated with liver fibrosis, can induce rather than protect from AIH [90]. Repetitive intraperitoneal injection of Sb and Bc for up to 8 weeks resulted in hepatic damage, with moderately elevated serum aminotransferase levels, hypergammaglobulinemia, generation of ANA, as well as appearance of clusters of cellular infiltrates in the liver [90]. It might very well be that herbal toxins are causing moderate but continuous damage to the liver and thereby initiate an inflammatory milieu that promotes chronic AIH-like disease.

7. Animal models: transgenic models developing spontaneous AIH-like disease

The inception of transgenic mice that either overexpress specific proteins under systemic or organ-specific promotors or are deficient in one or more proteins (KO mice) led to a multitude of novel AIH models and revealed new findings related to the immunopathology of AIH. There are two flavors of transgenic AIH models. First, models with inherent (transgenic) defects in immune regulation, such as the overexpression of certain pro-inflammatory factors or the absence/deficiency of immunoregulatory components, that result in a spontaneous development of AIH-like disease. Second, inducible models that carry transgenic target autoantigens in the liver and/or possess T cells that express a transgenic TcR specific for a liver autoantigen.

One of the most dramatic spontaneous AIH models is the NTxPD-1 −/− mouse. Such mice lack the programmed cell death 1 (PD-1) gene and are furthermore thymectomized (NT) [91]. As in regular PD-1-deficient mice NTxPD-1 −/− mice lack a sufficient number of regulatory T cells to maintain a normal T cell balance. The additional removal of the thymus resulted in the development of fatal AIH-like hepatitis characterized by T cell infiltrations into the liver parenchyma, a massive lobular necrosis, and the generation of ANA [91]. Administration of dexamethasone (Dex) or splenectomy prevented the development of fatal AIH in NTxPD-1 −/− mice [92]. However, whereas Dex did not prevent the production of aggressive T cells and Dex removal led to a relapse of AIH, splenectomy persistently reduced AIH-like disease indicating that the spleen is required for a continuous supply of aggressive T cells [92]. The finding that blockade of CXCL9 suppressed the progression of fatal AIH in the NTxPD-1 −/− mouse by decreasing the frequency of CXCR3+ T cells in the liver [93], suggests that chemokines, such as CXCL9, are important in orchestrating T cell trafficking from the spleen to the liver. Another spontaneous model has been generated by using triple knock-out mice deficient in Tyro3, Axl, and Mer receptor tyrosine kinases involved in the negative regulation of TLR-mediated intracellular signaling pathways [94]. In these mice an excessive TLR-dependent activation induces AIH-like disease characterized by persistently elevated serum aminotransferase levels, severe portal inflammation and piecemeal necrosis, and the generation of autoantibodies like ANA and SMA [94]. This model is based on earlier findings that TLR3 activation results in the release of pro-inflammatory cytokines (IFN-α and TNF-α) that induce CXCL9 and CXCL10 expression, which are two key chemokines orchestrating T cell infiltration of the liver [95].

A rather well-known knock-out line is the autoimmune regulator (Aire) deficient mouse. These mice have been used to identify mechanisms involved in the establishment of central tolerance in the thymus. Interestingly, Aire deficient humans and mice develop spontaneous autoimmune reactions against multiple target organs including the liver. In fact, patients with an Aire-deficiency, who suffer from autoimmune polyendocrine syndrome type 1 (APS-1), approximately 20% acquire AIH [96]. In context with the generation of an AIH model, it is interesting that a subgroup of Aire-deficient mice displays AIH-like features including lymphoplasmatic and periportal infiltrates, elevated aminotransferases and generate autoantibodies to several liver-specific and unspecific antigens [97]. Remarkably, the genetic background had a strong influence on the severity of AIH in Aire-deficient mice as AIH was less severe in mice with C57BL/6 background than in mice with a Balb/c background. Aire-deficiency in context with the autoimmunity-prone NOD/Ltj background was surprisingly also milder than in Aire −/− Balb/c mice [97]. Similar to Aire-deficient mice, conditional Traf6ΔTEC mice lacking the E3 ubiquitin protein ligase TRAF6 specifically in the thymic epithelial cells (TEC) possess an impaired negative selection of T cells. In contrast to Aire-deficient mice, Traf6ΔTEC mice develop a rather narrow spectrum of autoimmune reactivity affecting predominantly the liver [98]. They display histological and immunological features of human AIH, including interface hepatitis with cellular infiltrates of the liver parenchyma and the generation of ANA and anti-SLA antibodies [98,99]. Transfer of T cells isolated from Traf6ΔTEC mice into immunodeficient recipients also resulted in the development of AIH-like disease, indicating that without proper negative selection in the thymus aggressive T cells are released into the periphery that in the absence of additional peripheral regulatory mechanisms aggressively attack the liver [98].

8. Animal models: transgenic models requiring triggers to induce AIH-like disease

Most spontaneous transgenic AIH models are dependent on deficiencies in either induction or maintenance of general or liver-specific immune tolerance mechanisms. However, although risk factors, such as the HLA haplotype, mediate a certain susceptibility for AIH in humans, with exception of AIH in context with APS-1, no other immune deficiencies have been detected for the majority of patients with AIH. Thus, many attempts to generate a model for AIH were based on the concept of pathogen infection as a trigger of autoimmune diseases [62,100]. In many models, disease-related or unrelated autoantigens are expressed in the liver as defined targets for a liver-specific autoimmune process. In order to break tolerance to such target autoantigens, the animals are infected by a mostly liver-tropic pathogen that causes a local inflammatory burst in the liver. Thereby, central tolerance induction can be circumvented by an additional introduction of T cells isolated from transgenic animals expressing a target-autoantigen-specific TcR. Alternatively, the breakdown of tolerance can be facilitated by using pathogens expressing epitopes with a structural similarity to the target autoantigen. This concept of molecular mimicry is considered one possibility of how pathogen infection might be involved in the etiology of autoimmune diseases in susceptible individuals [2,61].

In the early 1990s, Frank Chisari's group generated transgenic mice expressing the hepatitis B virus surface antigen (HBsAg). An expression restricted to the liver was ensured by using a mouse albumin promoter. In order to break tolerance to HBsAg an adoptive transfer of activated T cells from HBsAg-primed donor mice was required [101,102]. In this model, HBsAg-specific cytotoxic T-lymphocytes (CTL) triggered apoptosis of hepatocytes expressing HBsAg and released IFN-γ upon antigen encounter. However, the observed hepatic injury was only transient and resembled a delayed-type hypersensitivity reaction rather than human AIH [101]. These data indicated that inducing chronic autoimmunity in the liver might be more difficult than in other organs, such as the pancreas. Breakdown of tolerance to autoantigens in the β-cells of the islet of Langerhans can be easily achieved in models like the RIP-LCMV-GP mouse, which express the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) in the β-cells. Upon infection with LCMV the mice develop T1D within 10–14 days [103,104]. Indeed, Ferber et al. demonstrated that a target autoantigen expressed solely on hepatocytes could lead to T cell tolerance by deletion, anergy, and receptor downregulation rather than autoimmunity [105]. They used, CRP-Kb mice that express the mouse MHC class I alloantigen H-2Kb under the inducible carbon-reactive protein (CRP) promoter and found that low levels of H-2Kb expression on hepatocytes induced tolerance to allogeneic skin grafts in CRP-Kb mice even when crossing CRP-Kb mice with mice that transgenically express H-2Kb-reactive TcRs [105]. These data are similar to the findings by Luth et al. that demonstrated that

ectopic expression of MBP protects mice from MS-like disease by promoting the generation of MBP-specific regulatory T cells (see above) [74]. The model autoantigen H-2Kb has been further used to demonstrate that the tolerogenic milieu in the liver can only be broken by an additional strong cytokine burst. Limmer et al. used double transgenic TcRxAlb.K^b mice expressing H-2Kb in hepatocytes and a H-2Kb-specific TcR on T cells. Transient hepatic damage characterized by elevated serum aminotransferase levels and cellular infiltration of the liver was induced by either infecting the mice with the liver tropic pathogen L. *monocytogenes*, by transfer of tumor cells expressing both H-2Kb as well as IL-2 [106], or by repetitive injection of immunostimulatory CpG-rich oligodeoxynucleotides (CpG-ODN) triggering the innate immune system via TLR9 [107]. Interestingly, termination of CpG-ODN administration abrogated the disease indicating that a chronic inflammatory stimulus was required for the self-perturbation of autoimmunity [107]. A further model relying on the simultaneous expression of the target autoantigen H-2Kb in the liver and a H-2Kb-specific TcR on T cells was generated by Bertolino et al. In order to circumvent central tolerance, they used thymectomized Met-Kb mice that express H-2Kb under the metallothionein (Met) promoter. These Met-Kb mice were then engrafted with non-transgenic thymus and reconstituted with bone marrow from H-2Kb-specific TcR transgenic mice [108]. Such mice showed transient hepatitis with cellular infiltrates peaking at 3 to 5 weeks after bone marrow reconstitution. Interestingly, the number of H-2Kb-specific T cells was reduced in the periphery of Met-Kb mice compared to non-transgenic control mice indicating the presence of regulatory control mechanisms. Indeed, the authors could demonstrate that although hepatocytes expressing H-2Kb efficiently primed naïve CD8 T cells, they failed to promote survival signals, such as IL-2 and bcl-xl induced by CD28 interaction, resulting in T cell death by neglect [109]. Later, it has been found that in Met-Kb mice injected with T cells expressing H-2Kb-specific TcR structural changes occur in LSEC at the peak of the transient hepatitis, which are associated with an attenuation of the disease [110].

Another target autoantigen has been used by Derkow et al. who generated two different transgenic mice expressing ovalbumin (OVA) either under the transferrin promoter (TF) or the apical sodium-dependent bile transporter promoter (ASBT) specifically in hepatocytes (TF-OVA mice) or cholangiocytes (ASBT-OVA mice), respectively [111]. Upon adoptive transfer of TcR-transgenic CD8 (OT-I) or CD4 (OT-II) T cells, OT-I, but not OT-II, T cells migrated to the liver in both TF-OVA as well as ASBT-OVA mice. However, although OT-I T cells proliferated and caused local inflammation and acute liver injury, the detected elevation of serum aminotransferases and cellular infiltration lasted only for 1–2 weeks [111]. As in the model using H-2Kb as target and TcR transgenic H-2Kb-specific effector T cells, the TF-OVA/ASBT-OVA models seem to lack a chronic inflammatory driver to maintain the autoaggressive immunity in the liver. Thus, Voehringer et al. used a similar approach as in the RIP-LCMV model for T1D mentioned above. They generated transgenic mice which expressed the immunodominant CD8 T cell epitope GP33 of the GP of LCMV under the albumin promoter (Alb) in the liver [112]. In addition to the transfer of TcR transgenic GP33-specific T cells they also infected the mice with LCMV and realized a transient hepatitis with elevated serum aminotransferase levels and cellular infiltrates in the liver. Unfortunately, the increase in serum aminotransferase levels lasted for only 12 days after infection / transfer. Interestingly, transfer of autoantigen-specific T cells or LCMV-infection alone did not induce disease [112]. These experiments again underline the tolerogenic nature of the liver, since to induce T1D in RIP-LCMV-GP mice only an infection with LCMV, but not an additional transfer of GP33-specific T cells is required [103,104]. In addition, the use of LCMV is critical for generating a model for AIH, since liver-tropic strains of LCMV cause a T cell-dependent liver disease with a transient serum aminotransferase level elevation that by histology resembles acute hepatitis B virus infection rather than AIH [113]. The nature of the autoantigen in such TcR/target antigen double-transgenic models seems to be critical for the outcome of the disease. Zierden et al. used instead of LCMV-GP33 or OVA, the influenza virus hemagglutinin (HA) as target antigen in the liver and generated double transgenic mice that in addition contained CD8 T cells expressing HA-specific TcR. Such Alb-HA/CL4-TCR mice displayed a spontaneous persistent liver inflammation not requiring infection with a liver-tropic pathogen [114]. However, the fact that most of the infiltrating HA-specific CD8 T cells displayed impaired proliferative capabilities with only little effector function as well as the presence of a considerable number of regulatory CD4 T cells suggests that immunosuppressive mechanisms also control the aggressiveness of the anti-HA response [114]. Dywicki et al. modified the Alb-HA in order to generate an inducible model with HA as target neoantigen. They generated Rosa26-HA mice in which the liver-specific HA expression was induced by infection with a recombination-deficient adenovirus expressing Cre recombinase [115]. For tracking the autoaggressive immune response to HA they transferred HA-specific TcR transgenic T cells. Surprisingly, although HA expression was in context of an inflammatory virus infection and a very high number of HA-specific T cells have been transferred, the mice did not develop AIH-like disease [115]. These data support the initial finding by Zierden et al., that after transfer HA-specific T cells acquired an anergic state with a low proliferative activity.

Besides an infection of animals with liver-tropic pathogens, an acute and/or chronic inflammatory milieu in the liver has also been generated by direct in situ expression of inflammatory factors, such as cytokines. Djilali-Saiah et al. used TTR-LCMV mice, which express the nucleoprotein (NP) of LCMV as a target antigen expressed under the control of the transthyretin (TTR) promoter in the liver. By DNA-vaccination with plasmids encoding for NP as well as the pro-inflammatory cytokine IL-12 they could cause liver damage characterized by elevated serum aminotransferase levels and minor cellular infiltrations. Further, a NP-specific CTL response was detectable after 2 months and persisted up to 5 months post-vaccination. [116]. In a follow-up study they used the natural human AIH autoantigens formiminotransferase cyclodeaminase (FTCD) and CYP2D6 to replace the AIH-unrelated NP of LCMV as triggering antigen. DNA-vaccination with a plasmid encoding for the antigenic regions of FTCD and CYP2D6 as well as the N-terminal region of mouse CTLA-4 and IL-12 resulted in significant inflammation in the liver. A vaccination with a CTLA-4-CYP2D6-FTCD-plasmid without IL-12 had no significant impact, [117]. Thus, IL-12 indeed seems to be an important driver of the inflammatory response in the liver. This conclusion has been further confirmed by Tamaki et al. who showed that vaccination of wild type C57BL/6 mice with dendritic cells loaded with well-differentiated hepatocellular carcinoma cells (DC/Hepa1–6) followed by intraperitoneal injection of recombinant human IL-12 caused a liver-specific inflammation and the generation of liver autoantigen (*S*-100)-specific proliferative and cytotoxic immune responses. [118]. In adoptive transfer experiments DC/Hepa1–6 activated splenocytes proved to exhibit a pathogenic phenotype, but only if the non-vaccinated recipient mice had also been treated with IL-12 [118]. In another model IL-12 has been administered with the help of an adeno-associated viral vector (AAV). Considering the abovementioned models, it is surprising that wild type mice that have been administered with AAV-IL-12 only developed AIH-like disease characterized by persistent cellular infiltrations of the liver, hepatic fibrosis, elevated serum aminotransferase levels, hypergammaglobulinemia, as well as generation of ANA and anti-SMA antibodies [119]. Mechanistically, they found that although AAV-mediated IL-12 was short lived, it induced the persistent expression of endogenous IL-12 and IFNγ throughout the observation time of 60 days. Further, they found that the observed hepatic damage was predominantly caused by CD4 and CD8 T cells rather than NK, NKT or B cells [119]. The strength of this model is surely its simplicity, since neither a particular target and/or triggering autoantigen nor the presence of autoantigen-specific TcR transgenic T cells is required. However, the absence of a defined autoantigen as target and/or trigger comes also with the disadvantage of not being able to track and

quantify the autoantigen-specific immune response.

9. Animal models: Models using naturally occurring autoantigens

In order to get as close as possible to the autoaggressive immune response in human AIH and in addition being able to track and quantify autoantigen-specific immune cells, several AIH models use target and/or trigger autoantigens to which antibodies or T cells have been identified in patients with AIH. CYP2D6 is the immunodominant and possibly the best characterized autoantigen recognized by antibodies and T cells of patient with AIH-2 [25,26,45]. The CYP2D6 mouse model utilizes human CYP2D6 as a triggering antigen, which is delivered to the liver of wild type FVB or C57BL/6 mice by infection of an adenovirus encoding for human CYP2D6 (Ad-2D6) [120]. Since mice are lacking the human CYP2D6 the triggered autoreactivity targets the mouse Cyp homologues. Infection of mice with Ad-2D6 results in an acute hepatic inflammation that subsequently develops into chronic AIH-like disease characterized by an interface hepatitis with cellular infiltrates that exhibit CD4 and CD8 T cells, B cells, macrophages, dendritic cells, and neutrophils [121]. Further, the mice generate LKM-1-like autoantibodies that largely recognize similar CYP2D6 epitopes than AIH-2 patients [66]. T cell epitope mapping revealed the presence of one CD4 and three distinct CD8 T cell epitopes [122]. Expectedly, these T cell epitopes are different from those recognized by AIH-patients due to the different MHC molecules [17]. However, it is interesting that the T cell epitopes are located in regions of intermediate homology between the triggering human CYP2D6 and the target mouse Cyp homologues. This indicates that molecular mimicry rather than identity was required to break Cyp-epitope-specific tolerance [122]. This conclusion is supported with data from experiments with human CYP2D6 transgenic mice, which upon infection with Ad-2D6, develop an ameliorated form of AIH-like disease only [121]. In particular, the frequency of CYP2D6-specific T cells is massively reduced in human CYP2D6 transgenic mice [122]. These initial data from the CYP2D6 model identify the liver autoantigen-specific T cells as one of the main drivers of the AIH pathogenesis. In addition, it serves a proof of principle that an infection with a pathogen that confers molecular mimicry to a liver autoantigen is indeed able to elicit a liver-specific autoimmune disease. However, although a transfer of autoantigen-specific TcR transgenic T cells or the liver-specific overexpression of a particular pro-inflammatory cytokine was not required, an infection with a liver-tropic pathogen (i.e. the adenoviral CYP2D6 carrier) was needed to generate an inflammatory milieu for the chronic autoimmune destruction to be initiated and/or propagated. A subcutaneous injection of recombinant CYP2D6 emulsified in complete Freund's Adjuvant was not sufficient to cause AIH-like disease even though CYP2D6-specific B and T cells were generated [122]. It has been postulated that human autoimmune diseases occur in susceptible individuals after exposure to one or more environmental factors. Besides pathogen infection or neoantigen formation by drugs and xenobiotics, obesity might also contribute to an inflammatory milieu in the liver. In particular, patients with NAFLD or NASH seem to be prone to providing a fertile field for subsequent autoimmunity. Indeed, when mice with NAFLD are infected with Ad-2D6 the ensuing AIH-like disease is exacerbated and NAFLD mice exhibit enhanced cellular infiltrations, augmented hepatic fibrosis and a higher frequency of autoreactive T cells [55]. Further, the CYP2D6 model has been used to elucidate mechanisms involved in the persistent fibrosis associated with AIH. Upon Ad-2D6 infection HSC get chronically activated resulting in an increased deposition of extracellular matrix components like collagen I and III and elevated expression of αSMA predominantly in and underneath the liver capsule but also in the perivascular region [123]. Thereby, the route of Ad-2D6 injection was decisive for HSC activation and fibrosis development. Whereas an intraperitoneal administration caused pronounced subcapsular fibrosis and clustering of inflammatory monocytes, intravenous administration resulted in the recruitment of an increased number of NK cells, which prevented chronic HSC activation and fibrosis [123]. Besides CYP2D6, the target of LC-1 antibodies, FTCD, has been also used in an adenovirus infection model. Hardtke-Wolenski et al. infected different mouse strains with either Ad-2D6 or Ad-FTCD and demonstrated that after an initial transient acute hepatitis a chronic AIH-like disease was evolving [124]. However, in contrast to the CYP2D6 model, the presence of a genetic predisposition for autoimmunity was required, since the development of chronic AIH could only be observed in the immune regulation deficient NOD mouse, but not in mice with a wildtype background such as C57BL/6 or FVB [124]. Another model that is based on pathogen infection has been reported by Aparicio et al. who infected wildtype C57BL/6 mice with the mouse hepatitis virus A59 (MHV). Interestingly, MHV-infected mice generated autoantibodies to fumarylacetoacetat hydrolase (FAH), [125]. FAH is predominantly expressed in liver and kidney and MHV-infected mice indeed displayed features of AIH, including hypergammaglobulinemia, elevated serum aminotransferases, hepatic cellular infiltrations and antibodies to other liver autoantigens. An additional stimulation by the synthetic peptide adjuvant PADRE (Pan HLA-DR binding epitope) exacerbated the MHV-induced AIH-like disease [125].

10. Conclusions

Many models for human AIH have been generated (Table 1). Some with the goal to develop a model system that closely mimics the human disease and thereby be able to evaluate possible treatment, and some that were intended to provide a tool to investigate basic aggressive or regulatory immune mechanisms. Many of which have been identified and extensively characterized in models, such as the Con A hepatitis model or models using TcR-transgenic T cells that allow for proper quantification and tracking of liver autoantigen-specific T cells. Hence, inflammatory factors that are critical for acute and/or chronic forms of hepatitis as well as many tolerogenic processes that maintain a healthy immune balance in the liver are known today. Unfortunately, many models that have helped identifying mechanisms of liver immunity and/or liver tolerance have not been pursued after one or two initial publications. Interestingly, most publications report about the failure or success of a specific model in inducing AIH-like disease and many even identified critical factors driving the disease but have not aimed at a blockade of the immunopathogenesis. Therefore, it may not come a s surprise that current therapy of AIH still largely relays on glucocorticoids and cytostatic drugs. Another reason might be that many of the models used antigens that are not target of the aggressive immune system in patients. Further, some models used rather artificial ways of breaking or circumventing liver tolerance by using disease unrelated target autoantigens, TcR-transgenic T cells, or thymectomy. However, often this was not sufficient to induce chronic hepatitis resembling human AIH and an additional local inflammation had to be induced by either using liver-tropic pathogens or by locally overexpressing critical inflammatory factors, like IL-12. Therefore, in the future it will be important to evaluate novel therapeutic strategies in models that are closer to the human situation. Models like the CYP2D6 or FTCD mouse model use natural occurring liver autoantigens as targets. In addition, an acute inflammatory insult of the liver is caused by infection of a liver-tropic pathogen that carries molecules that share a structural similarity or identity with the target autoantigen. Thus, the way for breaking tolerance is feasible and reflects a possible mechanism by which AIH might be initiated and/or promoted in patients. In future models it will be also important to incorporate humanized mice that for example carry critical human liver autoantigens in combination with defined HLA-risk alleles. Such a combination would allow a presentation of the same critical T cell epitopes than in patients and thereby an investigating of how tolerance to “real-life” autoantigen epitopes is broken and how the specific T cell response is perpetuated.

Transparency document

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