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TOPIC HIGHLIGHT

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Aetiopathogenesis of autoimmune hepatitis

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Abstract

The histological hallmark of autoimmune hepatitis (AIH) is a dense portal mononuclear cell infiltrate that invades the surrounding parenchyma and comprises T and B lymphocytes, macrophages, and plasma cells. An unknown but powerful stimulus must be promoting the formation of this massive inflammatory cellular reaction that is likely to initiate and perpetuate liver damage. An autoimmune attack can follow different pathways to inflict damage on hepatocytes. Liver damage is likely to be orchestrated by $CD4^+$ T lymphocytes recognizing an autoantigenic liver peptide. To trigger an autoimmune response, the peptide must be embraced by an HLA class Ⅱ molecule and presented to naïve $CD4^+$ T helper (Th0) cells by professional antigen presenting cells, with the co-stimulation of ligand-ligand fostering interaction between the two cells. Th0 cells become activated, differentiate into functional phenotypes according to the cytokines prevailing in the microenvironment and the nature of the antigen, and initiate a cascade of immune reactions determined by the cytokines produced by the activated T cells. Th1 cells, arising in the presence of the macrophage-derived interleukin (IL) -12, secrete mainly IL-2 and interferon-gamma (IFN-γ), which activate macrophages, enhance expression of HLA class Ⅰ (increasing liver cell vulnerability to a $CD8^+$ T cell cytotoxic attack), and induce expression of HLA class Ⅱ molecules on hepatocytes. Th2 cells, which differentiate from Th0 if the microenvironment is rich in IL-4, produce mainly IL-4, IL-10, and IL-13 which favour autoantibody production by B lymphocytes. Physiologically, Th1 and Th2 antagonize each other. Th17 cells, a recently described population, arise in the presence of transforming growth factor beta (TGF-β) and IL-6 and appear to have an important effector role in inflammation and autoimmunity. The process of autoantigen recognition is strictly controlled by regulatory mechanisms, such as those exerted by $CD4^+CD25^+$ regulatory T cells, which derive from Th0 in the presence of TGF-β, but in the absence of IL-6. If regulatory mechanisms fail, the autoimmune attack is perpetuated. Over the past three decades different aspects of the above pathogenic scenario have been investigated. In particular, a defect in immunoregulation affecting $CD4^+CD25^+$ regulatory T cells (T-regs) has been demonstrated in AIH, particularly at diagnosis or during relapse. Advances in the study of autoreactive T cells have occurred mostly in AIH type 2, since the knowledge that CYP2D6 is the main autoantigen has enabled the characterization of both CD4 and CD8 T cells targeting this cytochrome. CD4 T cells from patients with type 2 AIH positive for the predisposing HLA allele *DRB1*0701* recognize seven regions of CYP2D6, five of which are also recognized by CD8 T cells. High numbers of IFN-γ producing CD4 T cells and CD8 T cells are associated with biochemical evidence of liver damage, suggesting a combined cellular immune attack.

Key words: Autoimmune hepatitis; Aetiopathogenesis; Lymphocyte; Cellular immune attack; Histocompatibility lymphocyte antigen

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INTRODUCTION

Autoimmune hepatitis (AIH) is an inflammatory liver disease, affecting mainly females, characterized by elevated serum transaminase activity, positive organ and non-organ specific autoantibodies, elevated IgG, and a histological picture of interface hepatitis. There are two types of AIH according to their serology: type 1 is characterized by anti-nuclear (ANA) and/or anti-smooth muscle (SMA) antibodies; type 2 by anti-liver kidney microsomal type 1 (anti-LKM-1) antibody. The aetiology of AIH is unknown, though both genetic and environmental factors are involved in its expression.

Immune reactions against host liver antigens are believed to be the major pathogenic mechanism.

GENETICS

AIH is a "complex trait" disease, i.e. a condition not inherited in a Mendelian autosomal dominant, autosomal recessive, or sex-linked fashion. The mode of inheritance of a complex trait disorder is unknown and involves one or more genes, operating alone or in concert, to increase or reduce the risk of the trait, and interacting with environmental factors.

Susceptibility to AIH is imparted by genes within the major histocompatibility complex (MHC) - the human leukocyte antigen (HLA) region - on the short arm of chromosome 6, especially genes encoding HLA *DRB1* alleles. Since the role of class Ⅱ MHC molecules is to present peptide antigens to CD4 T cells, HLA class Ⅱ antigen presentation with ensuing T cell activation is likely to be involved in the pathogenesis of AIH.

In Europe and North America, susceptibility to AIH type1 is conferred by the presence of HLA *DR3* (*DRB1**0301) and *DR4* (*DRB1**0401), both heterodimers containing a lysine residue at position 71 of the *DRB1* polypeptide and the hexameric amino acid sequence LLEQKR at positions 67-72[1,2]. In Japan, Argentina, and Mexico, susceptibility is linked to *DRB1**0405 and *DRB1**0404, alleles encoding arginine rather than lysine at position 71, but sharing the motif LLEQ-R with *DRB1**0401 and *DRB1**0301[3]. Thus, K or R at position 71 in the context of LLEQ-R may be critical for susceptibility to AIH, favouring the binding of autoantigenic peptides, complementary to this hexameric sequence. However, an alternative model based on valine/glycine dimorphism at position 86 of the DR-β polypeptide has been proposed, better representing the key HLA associations in patients from Argentina and Brazil[1,2]. In a study from Japan, patients with AIH type 1 were found to have *DRB1* alleles which encode histidine at position 13[1,2]. There appears therefore to be at least three different models, suggesting that different genetic associations are present in different populations and that the peptides presented by HLA class Ⅱ molecules to the T cell receptors are different and may be derived from different antigens. Thus, these HLA associations may be the molecular footprints of the prevailing environmental triggers that precipitate AIH type 1 in different environments, though at the effector level the same autoantigenic target would be recognized. In this context, it is of interest that in South America presence of the HLA *DRB1**1301 allele, which predisposes to paediatric AIH type 1 in that population, is also associated with persistent infection with the endemic hepatitis A virus.

The lysine-71 and other models for AIH type 1 cannot explain completely the disease, since for example in European and North American patients presence of lysine-71 is associated with a severe, mainly juvenile, disease in those *DRB1**0301 positive, but to a mild, late onset, disease in those *DRB1**0401 positive. Other genes within or/and without the MHC are, therefore, likely to be involved in determining the phenotype. Possible candidates are the MHC encoded complement and tumour necrosis factor α genes, that are located in the class Ⅲ MHC region, and the MHC class I chain-related (MICA) A and B genes.

Susceptibility to AIH type 2 is conferred by HLA DR7 (*DRB1*0701*) and DR3 (*DRB1*0301*); patients positive for *DRB1*0701* have a more aggressive disease and worse outcome[4].

In an attempt to define additional susceptibility genes, a genome-wide approach was applied to a Japanese cohort of patients with AIH type 1[5]. This study found that 2 microsatellite markers (on chromosomes 11 and 18) out of 400 studied are associated with AIH type 1, though no protein of clear relevance to the disease is encoded in proximity of these two markers. The use of a larger number of microsatellites may prove more informative.

A form of AIH resembling AIH type 2 affects some 20% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a condition also known as autoimmune polyendocrine syndrome 1. APECED is a monogenic autosomal recessive disorder caused by homozygous mutations in the *AIRE1* gene and characterized by a variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis[6,7]. The *AIRE1* gene sequence consists of 14 exons containing 45 different mutations, with a 13 bp deletion at nucleotide 964 in exon 8 accounting for more than 70% of APECED alleles in the UK[6]. The protein predicted to be encoded by *AIRE1* is a transcription factor. *AIRE1* is highly expressed in thymic medullary epithelial cells and thymic stromal cells involved in clonal deletion of self-reactive T cells. Studies in a murine model indicate that the gene inhibits organ specific autoimmunity by inducing thymic expression of peripheral antigens in the medulla leading to central deletion of autoreactive T cells. Interestingly, APECED has a high level of variability in symptoms, especially between populations. Since various gene mutations have the same effect on thymic transcription of ectopic genes in animal models, it is likely that the clinical variability across human populations relates to environmental or genetic modifiers. Of the various genetic modifiers, perhaps the most likely to synergize with *AIRE* mutations are polymorphisms in the HLA region. HLA molecules are not only highly variable and strongly associated with multiple autoimmune diseases, but are also able to affect thymic repertoire selection of autoreactive T cell clones. Carriers of a single *AIRE* mutation do not develop APECED. However, although the inheritance pattern of APECED indicates a strictly recessive disorder, there are anecdotal data of mutations in a single copy of *AIRE* being associated with human autoimmunity of a less severe form than classically defined APECED[6,7]. A role of the heterozygote state for mutant *AIRE1* in the development of AIH remains

to be established. *AIRE1* mutations have been reported in 3 children with severe AIH type 2 and extrahepatic autoimmune manifestations[8].

IMMUNE MECHANISMS

The liver is regarded as a lymphoid organ with unique immunological properties[9]. Because of its location and function, the liver is continuously exposed to a large antigenic load that includes pathogens, toxins, tumour cells, dietary, and self-antigens. The liver contains large numbers of phagocytic cells, antigen presenting cells (APC) and lymphocytes and is a site for the abundant production of cytokines, complement components and acute phase proteins. The intrinsic lymphocyte population mainly resides in the portal tracts but is also scattered throughout the parenchyma, consisting of both cells of the innate (natural killer T cells, natural killer cells, and macrophages) and the adaptive (T and B cells) arms of the immune system. The blood entering the liver from the gut is rich in bacterial and dietary antigens that intermingle with lymphocytes. Immunoregulatory mechanisms are required to determine whether an antigen encounter will result in immunological unresponsiveness (tolerance) or reactivity. Liver autoimmunity implies loss of self-tolerance. Programmed cell death - apoptosis - which is responsible for the normal turnover of hepatocytes and the elimination of liver cells and unwanted lymphocytes in inflammatory pathologies is also relevant to the breakdown and/or maintenance of liver tolerance. First, death by apoptosis allows for non-inflammatory elimination of cell components in contrast to necrosis, which is pro-inflammatory and potentially autoantigenic. Second, apoptosis is the mechanism whereby the immune system is "cleansed" of autoreactive T and B lymphocytes as illustrated by the process of "activation induced cell death".

Various mechanisms have been proposed to account for the onset of an autoimmune liver response with no single initiating event being able to explain all instances of autoimmunity. Two general conditions, however, should prevail: self reactive B and T lymphocytes must exist in the immunological repertoire and autoantigens must be presented in conjunction with MHC class Ⅱ molecules by APC.

Humoral autoimmunity

Titres of antibodies to liver specific protein (LSP), a macromolecular complex present on the hepatocyte membrane[10], and to its well characterized components asialoglycoprotein receptor (ASGPR)[11] and alcohol dehydrogenase (ADH)[12] correlate with the biochemical and histological severity of AIH. Immunofluorescence studies on monodispersed suspensions of liver cells obtained from patients with AIH show that these cells are coated *in vivo* with antibodies reacting with antigens on the liver cell membrane[13]. A pathogenic role for these autoantibodies has been indicated by cytotoxicity assays demonstrating that autoantibody-coated hepatocytes from patients with AIH are killed when incubated with autologous or allogeneic lymphocytes[13]. The effector cell was identified as an Fc receptor positive mononuclear cell, presumably a natural killer (NK) cell.

In AIH type 2 the target of the disease-defining antibody, anti-LKM-1, is CYP2D6, a member of the hepatic P450 cytochrome family. Since CYP2D6 is expressed on the membrane of the hepatocytes and readily "accessible"[14], anti-LKM-1 antibodies might well have a pathogenic effect. In AIH type 2 anti-LKM-1 antibodies recognize linear regions (autoepitopes) of CYP2D6 in a hierarchical manner. Thus, the principal linear B-cell epitope, $CYP2D6_{193-212}$ is recognized by 93% of patients, $CYP2D6_{257-269}$ by 85%, $CYP2D6_{321-351}$ by 53%, and two additional minor epitopes $CYP2D6_{373-389}$ and $CYP2D6_{410-429}$ are recognized by 7% and 13% respectively[15]. Intriguingly, anti-LKM-1 antibodies are also found in some 5% of patients with hepatitis C virus (HCV) infection, among whom they appear to correlate with increased disease severity and adverse reactions to interferon α treatment[16]. The major CYP2D6 epitope recognized by patients with AIH type 2, $CYP2D6_{193-212}$, is also recognized by 50% of patients with anti-LKM-1 positive HCV infection[15]. Interestingly, these patients have antibodies that cross-react with homologous regions of HCV ($NS5B\ HCV_{2985-2990}$) and CYP2D6 ($CYP2D6_{204-209}$), and also of cytomegalovirus (exon $CMV_{130-135}$)[15]. Cross-reactive mechanisms to explain the emergence of CYP2D6 specific autoimmunity have also been suggested for other sequences of CYP2D6 which share homologies with HCV and herpes simplex virus (HSV), such as the sequence spanning aa 310-324 of E1 HCV and aa 156-170 of IE175 HSV1, which share homology with the CYP2D6 region comprising aa 254-271. As anti-LKM-1 antibodies cross-react with homologous regions of CYP2D6, HCV, HSV, and CMV, a "multi-hit" mechanism for the generation of these antibodies and possibly of AIH type 2 may be envisaged. In this model, multiple exposures to CMV or HSV, common viral pathogens, may establish permissive immunological conditions, by priming a cross-reactive subset of T cells, in a genetically predisposed host. Depending on the degree of immunological priming, i.e. level of exposure and the degree of genetic susceptibility (particularly at the HLA locus and coding regions for "innate" components of immunity), a minority of recurrently infected individuals may progress to autoimmune disease. It is therefore conceivable that an as yet unsuspected virus infection may be part of the origin of the autoimmune attack in AIH; this is to some degree in agreement with the concept expressed by Rolf Zinkernagel that an autoimmune disease is a viral disease in which the virus is unknown[17].

Molecular mimicry

The central function of the adaptive immune system is to generate T and B lymphocytes that can specifically recognize a potentially infinite number of non-self antigens without any prior information as to their structure. This is achieved by randomly generating a

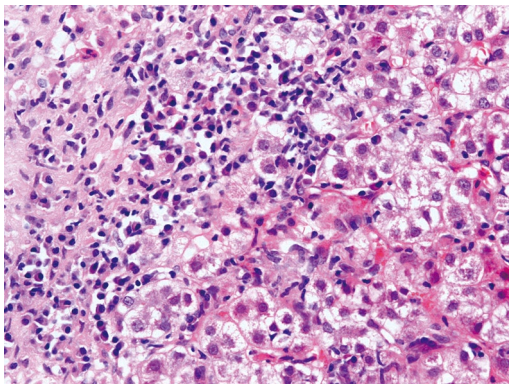

Figure 1 The portal and periportal inflammatory infiltrate characteristic of autoimmune hepatitis is composed by lymphocytes and plasma cells (interface hepatitis) (HE, × 40, provided by Dr. Alberto Quaglia).

large number of T and B cell specificities (*via* their respective antigen receptors-the T cell receptor and the antibody receptor) that are then able to clonally expand and recruit effector mechanisms on recognition of their cognate antigen or epitope. It is however, becoming clear that even this versatile system cannot cope with the extent of non-self antigenic diversity, and in the past decade convincing evidence has emerged for cross reactivity as an inherent property of immune ontogeny[18]. This has been studied primarily in the context of T lymphocytes, where it is clear that altered peptide ligands (APLs) - peptides similar in structure to the peptide antigen which are initially encountered - are able to induce both stimulatory and inhibitory T cell responses and, indeed, endogenous APLs operate in selecting the T cell repertoire in the thymus. This implies that a single T cell, rather than responding to a single antigen specificity, is able to cross-reactively respond to a number of antigens, thus expanding the antigenic specificities of the immune system to a level that reflects the antigenic diversity of the external environment[19].

This inherent potential for cross-reactivity, whilst allowing efficient responses to a vast array of pathogens also provides the immune system with the potential to cross-react with self, leading to autoimmunity. This process has been termed "molecular mimicry" as described above, whereby immune responses to external pathogens become directed towards structurally similar self components. Molecular mimicry has been shown to participate to the pathogenesis of autoimmune disease both in experimental models and in the human setting at the level of both T and B cells[18].

Cellular autoimmunity

The histological picture of interface hepatitis (Figure 1), with its striking infiltrate of lymphocytes, plasma cells, and macrophages was the first to suggest an autoaggressive cellular immune attack in the pathogenesis of AIH. Whatever is the initial trigger, this massive recruitment of activated inflammatory cells is likely to cause damage. Immunohistochemical studies have identified a predominance of T lymphocytes mounting the α/β T cell receptor[20]. Amongst the T cells, a majority are positive for the CD4 helper/inducer phenotype, and a sizeable minority for the CD8 cytotoxic phenotype. Lymphocytes of non-T cell lineage are fewer and include (in decreasing order of frequency) natural killer cells (CD16/CD56 positive), macrophages, B cells, and plasma cells. The involvement of natural killer T cells is the focus of ongoing studies.

There are different possible pathways that an immune attack can follow to inflict damage on hepatocytes (Figure 2) as discussed below.

Impairment of T regulatory cells

An impairment of immunoregulatory mechanisms, which would enable the autoimmune response to develop, has been repeatedly reported in the setting of both human and experimental autoimmunity. Thus, in early studies it was shown that patients with AIH have low levels of circulating T cells expressing the CD8 marker[21], and impaired suppressor cell function which segregates with the possession of the disease-predisposing HLA haplotype B8/DR3[22] and is correctable by therapeutic doses of corticosteroids[23]. Furthermore, patients with AIH have been reported to have a defect specifically in a subpopulation of T cells that control the immune response to an as yet unidentified liver-specific membrane antigen(s)[24]. Recent experimental evidence confirms an impairment of the immunoregulatory function in AIH. Thus, among recently defined T cell subsets with potential immunosuppressive function, $CD4^+$ T cells constitutively expressing the interleukin 2 receptor α chain (CD25) (T-regulatory cells, T-regs) have emerged as the dominant immunoregulatory lymphocytes[25]. These cells, which in health represent 5%-10% of the total population of peripheral $CD4^+$ T cells, control the innate and the adaptive immune responses by preventing the proliferation and effector function of autoreactive T cells. Their mechanism of action involves mainly a direct contact with the target cells, and to a lesser extent the release of immunoregulatory cytokines, such as interleukin 10 and transforming growth factor β 1 (TGF-β). In addition to CD25, which is also present on T cells undergoing activation, T-regs express a number of additional markers such as the glucocorticoid induced tumour necrosis factor receptor, CD62L, the cytotoxic T lymphocyte associated protein-4 (CTLA-4) and the forkhead/winged helix transcription factor FOXP3, the expression of which is closely associated with the acquisition of regulatory properties. In patients with AIH, T-regs are defective both in number and function compared to normal controls and these abnormalities relate to the stage of disease, being more evident at diagnosis than during drug-induced remission[26-28]. The percentage of T-regs inversely correlates with markers of disease severity, such as levels of antibodies to anti-soluble liver antigen[29] and anti-LKM-1 autoantibody titres, suggesting that a reduction T-regs favours the serological manifestations of autoimmune liver disease. If loss of immunoregulation was central to the pathogenesis of autoimmune liver disease, treatment should concentrate on restoring T-regs ability to expand, with consequent

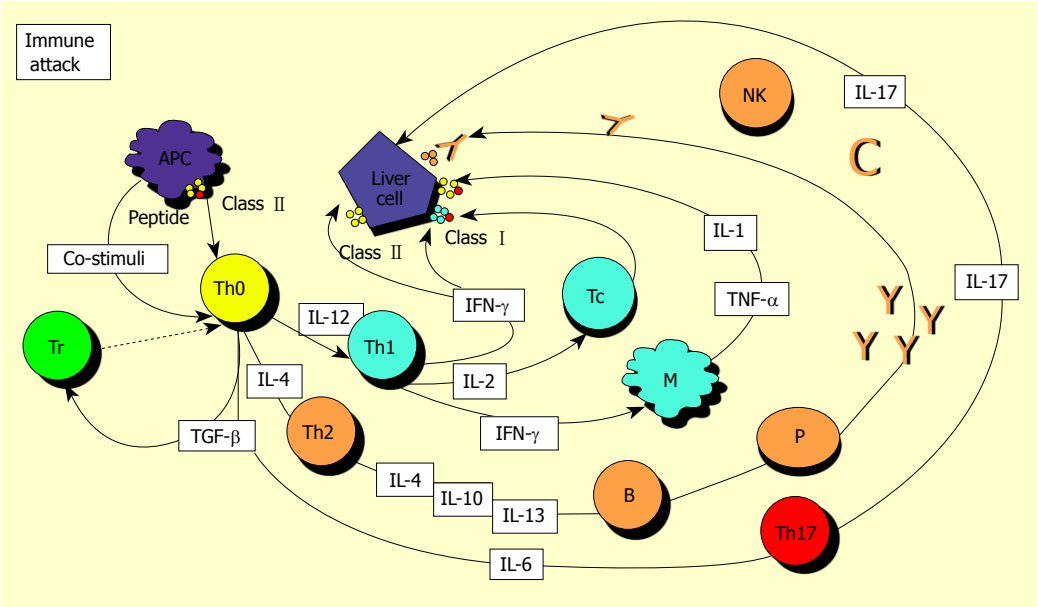

Figure 2 Autoimmune attack to the liver cell. A specific autoantigenic peptide is presented to an uncommitted T helper (Th0) lymphocyte within the HLA class II molecule of an antigen-presenting cell (APC). Th0 cells become activated and, according to the presence in the microenvironment of IL-12 or IL-4 and the nature of the antigen, differentiate into Th1 or Th2 and initiate a series of immune reactions determined by the cytokines they produce: Th2 secrete mainly IL-4, IL-10 and IL-13, and direct autoantibody production by B lymphocytes; Th1 secrete IL-2 and IFN-γ, which stimulate T cytotoxic (Tc) lymphocytes, enhance expression of class I and induce expression of class II HLA molecules on hepatocytes and activate macrophages; activated macrophages release IL-1 and tumour necrosis factor alpha (TNF-α). If regulatory T cells do not oppose, a variety of effector mechanisms are triggered: liver cell destruction could derive from the action of Tc lymphocytes; cytokines released by Th1 and recruited macrophages; complement activation or engagement of Fc receptor-bearing cells such as natural killer (NK) lymphocytes by the autoantibody bound to the hepatocyte surface. The role of the recently described Th17 cells, which arise in the presence of transforming growth factor beta (TGF-β) and IL-6, is under investigation.

increase in their number and function. This is at least partially achieved by standard immunosuppression, since T-regs numbers do increase during remission[26,28].

CD4 autoreactive T cells

To trigger an autoimmune response, a peptide must be embraced by an HLA class Ⅱ molecule and presented to uncommitted T helper (Th0) cells by professional APC, with the co-stimulation of ligand-ligand (CD28 on Th0, CD80 on APC) interaction between the cells (Figure 2). Once the autoimmune response has been initiated and in the absence of effective immunosuppressive treatment, tissue damage ensues and persists. In an inflammatory milieu, hepatocytes from patients with AIH, in contrast to normal hepatocytes, express HLA class Ⅱ molecules[20], as well as class Ⅰ. Although lacking the antigen processing machinery typical of APC, MHC-class Ⅱ -bearing hepatocytes may present peptides through a bystander mechanism[30]. In the presence of impaired immunoregulation and inappropriate expression of HLA class Ⅱ antigens on the hepatocytes, an intracellular autoantigenic peptide from intact hepatocytes could be presented to the CD4 helper/inducer T cells leading to their activation. Although no direct evidence exists as yet that an autoantigenic peptide is in fact presented by MHC-class Ⅱ-bearing hepatocytes and recognized by CD4 T helper cells, activation of such cells has been documented in AIH[21]. Circulating T cells specific for liver autoantigens are found also in normal subjects, but in AIH their frequency is at least 10-fold higher[31]. This finding suggests that the pool of liver-autoreactive T cells undergoes a significant expansion in patients with AIH and hence may be involved in the initiation and perpetuation of the immune attack to the liver.

Given that T cells recognize antigens in a precise fashion, studies in the early 1990s were conducted at a single T cell level in order to characterize antigen-specific T cell recognition. T cell clones generated from the peripheral blood were mainly $CD4^+$ α/β T cells[32], while a large proportion of liver-derived clones were either $CD4/CD8^-$ γ/δ or $CD8^+$ α/β T cells[31,33,34]. Both α/β and γ/δ T cell clones proliferated in the presence of a crude liver membrane preparation, liver specific protein and asialoglycoprotein receptor, α/β being more reactive than γ/δ clones. Some of the liver membrane reactive clones also proliferated in the presence of LSP and/or ASGPR, responded in an HLA class Ⅱ restricted fashion and helped autologous B cells to produce immunoglobulins, and in particular autoantibodies to LSP and ASGPR[32].

T cell ligands are best studied in AIH type 2, since the target of anti-LKM-1 has been characterized as *CYP2D6*. *CYP2D6*$_{262\text{-}285}$ specific T cell clones generated from liver tissue and peripheral blood express a Th1 $CD4^+$ phenotype[33,34]. In contrast to the latter study that focused on a short antigenic sequence of CYP2D6, a systematic approach based on the construction of overlapping peptides covering the whole CYP2D6 molecule was recently adopted to define the specificity of *ex vivo* CYP2D6 reactive T cells in patients with AIH type

2[4]. This study showed that T cells from patients positive for the predisposing HLA allele *DRB1**0701 recognize in a proliferation assay seven regions of CYP2D6, four of which are also partially recognized by T cells of *DRB1**0701 negative patients. While distinct peptides induce production of IFN-γ, IL-4 or IL-10, peptides that induced IFN-γ and proliferative responses overlap. There was also an overlap between sequences inducing T and B cell responses. The number of epitopes recognized and the quantity of cytokine produced by T cells are directly correlated to biochemical and histological markers of disease activity. These results indicate that the T cell response to CYP2D6 in AIH type 2 is polyclonal, involves multiple effector types targeting different epitopes, and is associated with hepatocyte damage[4].

CD8 autoreactive T cells

In addition to the unfolding role of CYP2D6 specific CD4 T cells in AIH type 2, there is growing evidence implicating an HLA class I restricted CD8 response in the pathogenesis of autoimmune liver damage. In the early 1990s CD8 T cell clones specific for ASGPR were described in patients with AIH[32]. Recent studies have identified CYP2D6 specific CD8 T cells capable of secreting IFN-γ and of exerting cytotoxicity after recognition of CYP2D6 epitopic sequences in an HLA class I restricted fashion[35].

Taken together, the data presented above suggest that a failure of immune homeostatic processes, normally keeping the response against self-antigens under control, is involved in the pathogenesis of AIH. The prime mechanism for tolerance breakdown remains to be elucidated. There is some evidence that molecular mimicry mechanisms involving viral self-mimicking and autologous sequences may be involved[36,37] and such mechanisms are the focus of ongoing studies.

ANIMAL MODELS

Research on the pathogenesis of AIH has been hampered by the lack of animal models reproducing faithfully the human condition. The ideal model for AIH should have a well-defined initiating event followed by chronic inflammation leading to fibrosis. Recently, researchers have focused on animal models of AIH type 2, since in this condition the autoantigen is well defined. The model produced by the group of Alvarez[38] is based on immunizing every two weeks for three times C57BL/6 female mice with a plasmid containing cDNA for the antigenic region of human CYP2D6, which is the target of anti-LKM-1, and formimino-transferase cyclodeaminase, which is the target of anti-liver cytosol-1 and an additional marker for AIH type 2[39], together with the end of the terminal region of murine CTLA-4. The latter was added to facilitate antigen uptake by antigen presenting cells. In a parallel set of experiments a plasmid containing the cDNA encoding IL-12, a Th1 skewing pro-inflammatory cytokine, was also used. When autoantigens and IL-12 were used to break tolerance, antigen specific autoantibodies were produced, a relatively modest elevation of transaminase levels at 4 and 7 mo was observed, and a portal and periportal inflammatory infiltrate composed of CD4 and CD8 T cells and, to a lesser extent, B cells was demonstrated 8-10 mo after the third immunization. When the same immunization protocol was used in different mouse strains, either a mild hepatitis or no inflammatory changes were observed indicating the importance of a specific genetic background. Another model of AIH type 2 uses CYP2D6 transgenic mice and aims at breaking tolerance with an adenovirus-CYP2D6 vector[40]. While focal hepatocyte necrosis was seen in both mice treated with the adenovirus-CYP2D6 vector and control mice treated with adenovirus alone, only the former developed chronic histological changes, including fibrosis, reminiscent of AIH. The hepatic lesion was associated to a specific immune response to an immunodominant region of CYP2D6 and a cytotoxic T cell response to adenovirus-CYP2D6 vector infected target cells. Though these two experimental approaches provide useful information on the possible pathogenic mechanisms leading to human AIH type 2, a model that closely reproduces human AIH type 1 is still lacking, hampering the elucidation of pathogenic mechanisms in this form of AIH.

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