Xenobiotics and loss of tolerance in primary biliary cholangitis

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Abstract

Data from genome wide association studies and geoepidemiological studies established that a combination of genetic predisposition and environmental stimulation is required for the loss of tolerance in primary biliary cholangitis (PBC). The serologic hallmark of PBC are the presence of high titer anti-mitochondrial autoantibodies (AMA) that recognize the lipoyl domain of the mitochondrial pyruvate dehydrogenase E2 (PDC-E2) subunit. Extensive efforts have been directed to investigate the molecular basis of AMA. Recently, experimental data has pointed to the thesis that the breaking of tolerance to PDC-E2 is a pivotal event in the initial etiology of PBC, including environmental xenobiotics including those commonly found in cosmetics and food additives, suggesting that chemical modification of the PDC-E2 epitope may render its vulnerable to become a neo-antigen and trigger an immune response in genetically susceptible hosts. Here, we will discuss the natural history, genetics and immunobiology of PBC and structural constraints of PDC-E2 in AMA recognition which makes it vulnerable to chemical modification.

Key words: Antimitochondrial autoantibodies; Primary biliary cholangitis; Pyruvate dehydrogenase E2; Breaking of tolerance; Xenobiotics

Core tip: Environment influences immune functions. In this paper, we examine how environmental chemicals can trigger autoimmunity in an organ specific autoimmune disease, primary biliary cholangitis (PBC). PBC is liver specific autoimmune disease characterized by high titer of anti-mitochondrial autoantibodies directed against the E2 subunit of pyruvate dehydrogenase (PDC-E2) lipoyl domain. Here,

we present experimental evidence from quantitative structure-activity relationship and animal models that xenobiotic modification of the PDC-E2 lipoyl domain could lead to loss of self-tolerance and is a pivotal event in the initial etiology of PBC in genetically susceptible hosts.

INTRODUCTION

The loss of tolerance is a central theme in autoimmunity and genetics and geoepidemiological studies have reflected that environmental factors contribute to this breach of tolerance[1-10]. This thesis is exemplified in primary biliary cholangitis (PBC), a prototype organ specific autoimmune disease[11]. The mechanism of how immunological tolerance is broken in PBC is still enigmatic[12]. Importantly, the autoantigen recognized by AMA was first cloned in 1987 and subsequently identified as the E2 subunit of pyruvate dehydrogenase (PDC-E2)[13,14]. The epitopes of AMA have been mapped to the highly conserved lipoic acid binding domain of the 2-oxo acid dehydrogenases including PDC-E2, branched chain 2-oxo-acid dehydrogenases (BCOADC-E2), oxoglutarate dehydrogenase (OGDC-E2) and the E3 binding protein (E3BP)[13-16]. Extensive efforts in defining the target mitochondrial autoantigens, T and B cell epitopes, the innate and adaptive immune responses, the immunobiology of the biliary epithelium, and the pathology of biliary duct epithelial cell destruction have greatly advanced our knowledge of the molecular mechanisms in tissue damage[13,17-29]. This focus of this review is to provide a comprehensive view of our current understanding on the natural history, genetics and immunobiology of PBC with emphasis on experimental data that illustrate the loss of tolerance to PDC-E2 is a pivotal event in the etiology of PBC[25,30-32].

NATURAL HISTORY AND GENETICS OF PRIMARY BILIARY CHOLANGITIS

Primary biliary cholangitis (PBC), previously known as primary biliary cirrhosis[33] is a female predominant liver-specific autoimmune disease with middle-age onset. It has an average incidence of 2.7 cases per 100000[34], but epidemiological studies suggest that the incidence of PBC is increasing[35]. There is variation in the prevalence of disease between geographic locations[35,36]; PBC is more prevalent in Northern Europe, North America and Latin America and less common in Eastern Asia, Africa, and Australia[37,38]. Clinically, PBC is characterized by

the presence of high titer AMA and immune-mediated progressive destruction of biliary epithelial cells (BECs) within small bile ducts, eventually leading to cholestasis, fibrosis, and, potentially, liver cirrhosis[12]. Approximately 50%-60% of patients are asymptomatic at diagnosis. The disease has a long latency period[39,40], followed by the development of symptoms that may include fatigue, pruritus, cutaneous pigmentation and, later, bleeding varices, edema, or ascites[41]. The prognosis of patients diagnosed with PBC has improved significantly over the past two decades, perhaps because patients are being diagnosed earlier. PBC is a "model" autoimmune disease with significant literature on genetics, environment and animal models[17,25,33,42-51].

The female predominance among individuals with PBC suggests that there are significant genetic components in this disease, supported by the high frequency of X chromosome monosomy in patients with PBC[52,53] and Y chromosome loss in male patients with PBC[54]. Reports from recent genetic studies demonstrate that in addition to the MHC, several loci are associated with susceptibility to PBC, including interleukin (IL) 12-related pathways, SPIB, IRF5-TNPO3, and 17q12-2. The candidate genes identified by genome wide association studies include STAT4, DENND1B, CD80, IL7R, CXCR5, TNFRSF1A, CLEC16A, and NFKB1[55-58]. Data on familial clustering of PBC demonstrates that first-degree relatives of PBC patients have an increased risk of developing disease and most often these clusters involve mother-daughter pairs, consistent with the female preponderance of the disease[59-61]. Furthermore, twin studies have demonstrated a high concordance for PBC in monozygotic twins[62]. These studies provide evidence for a genetic basis underlying PBC. Genome analysis of DNA methylation, copy number variation and gene expression of monozygotic twins and sisters discordant for PBC have also indicated a contribution of epigenetic events[63]. However, environmental factors also play a role in the development of the disease[64], and multiple environmental components including chemicals[30,65-67] and bacteria[68-71] have been implicated.

IMMUNOLOGICAL FEATURES OF PRIMARY BILIARY CHOLANGITIS

AMA are present in over 95% of patients with PBC and are diagnostic of PBC[23]. The autoantigens of AMA have been identified as the E2 subunits of the 2-oxo-acid dehydrogenase complexes (2OADC-E2), including the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxo acid dehydrogenase complex (BCOADC-E2) and 2-oxo-glutarate dehydrogenase complex (OGDC-E2) within the inner mitochondrial matrix[13,15,16,72]. The E2 enzymes have a common structure consisting of an N-terminal domain containing a single or multiple lipoyl groups. Previous studies have demonstrated that the dominant epitopes
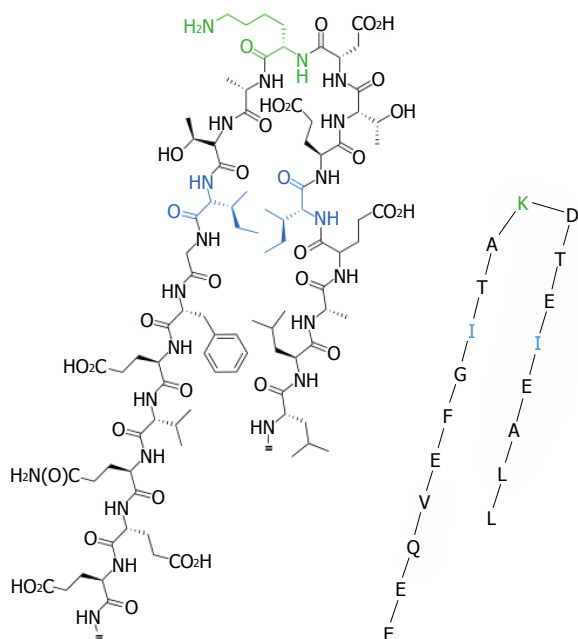
Figure 1 Schematic representation of the pyruvate dehydrogenase E2 lipoyl domain. Including the 19 residues (LLAEI-ETDKA-TIGFE-VQEE), lipoic acid is covalently attached to the ε group of lysine (K) *via* an amide bond.

recognized by AMA are all within the lipoyl domains of these target antigens[73].

In patients with PBC, Both CD4^{+} and CD8^{+} T cells are present in portal tracts, around damaged bile ducts, strongly suggesting the participation of cellular immune mechanisms in biliary damage[12]. PDC-E2 autoreactive CD4 T cells are present in peripheral blood and liver; there is a specific 100-150 fold increase in the number of PDC-E2-specific CD4 T cells in the hilar lymph nodes and liver versus peripheral blood in patients with PBC[27]. The PDC-E2 autoepitope for both CD4 and HLA class Ⅰ restricted CD8 T cells, overlaps with the B cell epitope, which spans the lipoyl domain[74]. Similar to CD4 autoreactive T cells, there is a 10-fold higher frequency of PDC-E2 specific CD8 T cells within the liver versus peripheral blood. Moreover, the precursor frequency of PDC-E2-specific autoreactive CD8 T cells is significantly higher in early rather than late stage of the disease[74]. Recent reports also substantiate the significance of innate immunity, including monocytes, toll like receptors and natural killer cells in the development of PBC[75,76]. The multi-lineage response to the PDC-E2, the immunodominant mitochondrial autoantigen in PBC, points to the thesis that loss of tolerance to PDC-E2 is the initiating event that leads to the subsequent development of clinical biliary pathology[12].

MOLECULAR MIMICRY OF LIPOIC ACID AND XENOBIOTICS IN PBC

Epidemiological and mechanistic studies on auto-immunity have strongly demonstrated the etiologic

contribution of environment[77], likely through molecular mimicry. Although microorganisms are possible candidates for the induction of autoimmune disease by molecular mimicry[78-84], there are other potential environmental factors, including chemical compounds foreign to a living organism. Examples include drugs, pesticides or other organic molecules that have the potential to modify host proteins and render them more immunogenic[77].

Halothane hepatitis is a xenobiotic-induced liver disease that occurs when susceptible individuals develop an immune response against trifluoroacetylated (TFA)-adduct protein. Exposure to TFA-conjugated self proteins results in antibody responses against such TFA-self proteins. Interestingly, anti-TFA also recognizes the lipoylated domain of PDC-E2[14,85]. The immunological cross-reactivity of anti-TFA antibodies with the immunodominant epitope in PBC prompted us to examine in depth molecular mimicry.

Site-directed mutagenesis of the PDC-E2 lipoyl domain demonstrated that AMA recognition is con-strained by respective amino acid sequence in epitope (Figure 1, Table 1)[86,87]. The uniqueness of epitope specificity of AMA within the lipoyl domains of the 2OADC-E2 enzymes in patients with PBC[87-89] suggests that the lipoic acid domain is likely a lynchpin to the etiology of PBC. High resolution structural analysis and modeling studies of the PDC-E2 lipoyl domains from both prokaryotes and eukaryotes demonstrates that lipoic acid is covalently attached to the ε group of lysine (K) *via* an amide bond and is prominently displayed on the outer surface of PDC-E2. More importantly, the ability of lipoic acid to rotate by means of its "swinging arms" with respect to the bulk of the entire PDC-E2 molecule allows accessibility of its dithiolane ring for reduction acylation[90,91]. Although the change in conformation and the existence of multiple conformations of the lipoyl domain during reductive acetylation are important in catalyzing acyl transfer[90], it also renders PDC-E2 susceptible to aberrant chemical modifications.

Accumulating evidence implicates that the loss of tolerance to PDC-E2 is pivotal in the initiation event of PBC and that AMA specificities reflect aspects of the induction phase of the disease[11,25,31,39]. Indeed the role of environment is well-known in many autoimmune diseases[30,92-98].

We hypothesized that xenobiotic modification of the native lipoyl moiety of the major mitochondrial autoantigen PDC-E2, may lead to loss of self-tolerance and eventually biliary lesions (Figure 2)[99]. This thesis is based on the findings of (1) readily detectable levels of immunoreactivity of PBC sera against comprehensive panels of protein microarrays, which mimic the inner lipoyl domain of PDC-E2; and (2) subsequent quantitative structure-activity relationships. Data from quantitative structure-activity relationship (QSAR) analysis demonstrated that AMA-

Table 1 Serological reactivity of primary biliary cholangitis sera to the recombinant proteins of wild type pyruvate dehydrogenase E2 lipoyl domain, single amino acid mutants double, triple and quadruple mutants[1]

Mutant No.		Amino acid sequence	PBC sera[2]		Purified PBC IgG[3]
			IgG	IgM	
PDC-E2 wild type		LLAEIETDKATIGFEVQEE	1	1	1
Mutant 3		LLAEAETDKATIGFEVQEE	0.476 ± 0.029	0.504 ± 0.043	0.408 ± 0.052
Mutant 9		LLAEIETDKATAGFEVQEE	0.706 ± 0.029	0.781 ± 0.054	0.552 ± 0.065
Mutant 12		LLAEIETDKATIGFAVQEE	0.659 ± 0.034	0.768 ± 0.096	0.482 ± 0.074
Double amino acid substitution	Mutant 1	ALAEIETDKATAGFEVQEE	0.334 ± 0.029	0.253 ± 0.034	0.075 ± 0.023
	Mutant 2	ALAEIETDKATIGFAVQEE	0.461 ± 0.031	0.435 ± 0.045	0.663 ± 0.069
	Mutant 3	LLAEAETDKATAGFEVQEE	0.066 ± 0.009	0.093 ± 0.016	0.024 ± 0.007
	Mutant 4	LLAEAETDKATIGFAVQEE	0.111 ± 0.017	0.095 ± 0.016	0.043 ± 0.016
Triple amino acid substitution	Mutant 1	ALAEAETDKATAGFEVQEE	0.017 ± 0.004	0.044 ± 0.009	0.038 ± 0.017
	Mutant 2	LLAEAETDKATAGFAVQEE	0.019 ± 0.003	0.054 ± 0.012	0.050 ± 0.007
Quadruple amino acid substitution		ALAEAETDKATAGFAVQEE	0.024 ± 0.005	0.066 ± 0.012	0.075 ± 0.031

[1]16 single alanine substitution mutants along a peptide that constitutes the beta sheet of the PDC-E2 inner lipoyl domain, 4 double aa substitution mutants, 3 triple and one quadruple mutants were also constructed. Purified proteins from all these constructs were analyzed for Ig reactivity with PBC sera. 3/16 of the single amino acid mutants have much reduced antibody binding are shown. Other alanine substitutions have Ig reactivity similar to wild type PDC-E2. Control sera samples include (lupus, $n = 30$, Crohn's disease, $n = 20$, PSC, $n = 28$, scleroderma $n = 20$) did not react; [2]Relative ratio of serological IgG and IgM reactivity compared to wild type determined by ELISA at 1:4000 sera dilution ($n = 60$); [3]Relative ratio of purified IgG reactivity to wild type determined by ELISA ($n = 10$). PBC: Primary biliary cholangitis.
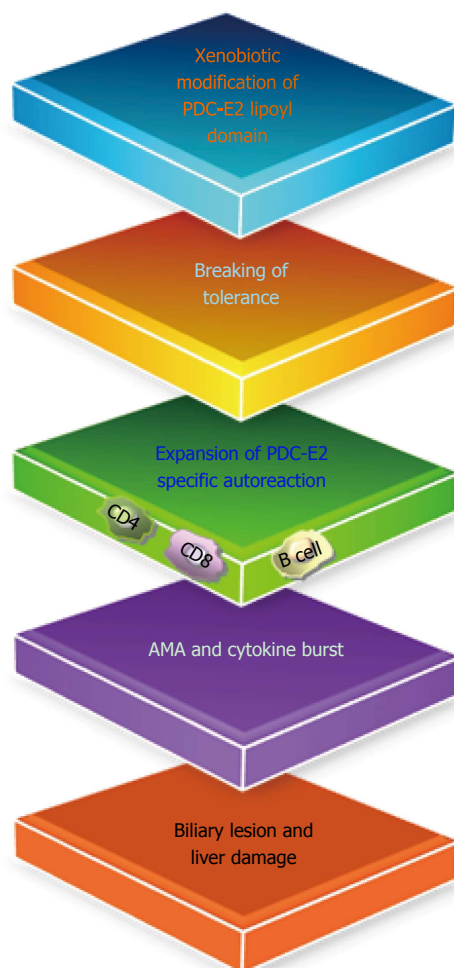

Figure 2 Xenobiotic modification of the native lipoyl moiety of the major mitochondrial autoantigen pyruvate dehydrogenase E2, lead to the loss of self-tolerance and eventually biliary lesions in primary biliary cholangitis. PDC-E2: Pyruvate dehydrogenase E2; AMA: Anti-mitochondrial autoantibody.

positive PBC sera, but not controls, reacted to a number of xenobiotic-modified PDC-E2 structures[66,100] with a particularly striking level of reactivity against 6,8-bis(acetylthio) octanoic acid (SAc)-PDC-E2[101]. Recent data further suggest that chemical modification of PDC-E2 lipoic acid, *via* an electrophilic attack on the lipoic acid disulfide bond, triggers loss of tolerance to PDC-E2[30,101,102]. Such modifications could substantially affect the conformation of the PDC-E2 lipoyl domain and its immunogenicity in genetically susceptible hosts. Importantly, one of these chemical compounds is 2-octynoic acid (2-OA), a chemical commonly found in cosmetics and food additives[66].

XENOBIOTICS INDUCED MODELS OF PBC AND THE CONTRIBUTIONS OF EFFECTOR PATHWAYS IN AUTOIMMUNE CHOLANGITIS

Interestingly, immunization of C57BL/6 mice and NOD.1101 (NOD.B6 *Idd10 Idd18*r2) mice with 2-OA coupled to BSA, but not BSA alone, induced high titer AMAs, portal inflammation, and autoimmune cholangitis similar to human PBC[103,104]. These models provide a persuasive argument in favor of an environmental origin for human PBC[81,103,105,106]. We further investigated the role of IL-12-Th1/IL-23-Th17 pathways in the development of autoimmune cholangitis in this PBC model by using specific cytokine knockout mice (Table 2)[18]. In particular, we constructed several unique gene-deleted mice, including C57BL/6 mice deleted in both Th1 and Th17 (IL-12p40), Th1 cytokine (IL-12p35, IFN-γ) or Th17 cytokine (IL-23p19, IL-17A, IL-

Table 2 Influence of Th1 and Th17 cytokine in liver pathology of 2OA-BSA induced primary biliary cholangitis mouse model

Pathway	Cytokine k/o	Liver pathology
Th1	IL-12p35$^{-/-}$	Reduced liver infiltrates, reduced bile duct damage
Th1	IFN-γ$^{-/-}$	Marked reduction in liver infiltrates, bile duct normal
Th1/Th17	IL-12/IL-23p40$^{-/-}$	Abolish autoimmune cholangitis
Th17	IL-23p19$^{-/-}$	Reduced liver infiltrates, reduced bile duct damage
Th17	IL-17A$^{-/-}$	Reduced liver infiltrates, reduced bile duct damage
Th17	IL-17F$^{-/-}$	Similar to positive control
Th17	IL-22$^{-/-}$	Reduced liver infiltrates, reduced bile duct damage

IFN-γ: Interferon-γ; IL: Interleukin; Th17: T helper 17.

17F or IL-22). We immunized each of these cytokine-deficient mice with 2-OA-BSA and followed the natural history of their immunopathology. Our data indicate that while both IL-12/Th1 and IL-23/Th17 are involved in cholangitis, it is the IL-12/Th1 signaling pathway that elicits liver pathology in this xenobiotic induction disease model of PBC. In fact, deletion of IFN-γ prevents disease and suppresses autoantibodies. Importantly, deletion of the Th17 cytokines IL-17A and IL-22, but not IL-17F, reduces biliary damage; IL-17A-knockout mice have also reduced levels of AMAs. We further demonstrated that the production of IFN-γ is significantly decreased in livers of IL-23p19$^{-/-}$, IL-17A$^{-/-}$ and IL-22$^{-/-}$ mice compared with controls. However, the ability of T cells to produce IFN-γ was not affected in Th17 cytokine-deficient mice. Thus, in the 2-OA-BSA immunized mice model: (1) Both IL-12/Th1 and IL-23/Th17 are involved in cholangitis; (2) IL-12/Th1 signaling pathway is critical in eliciting liver pathology; and (3) IL-23/Th17 pathway is involved in perpetuating the IL-12/IFN-γ mediated pathology. We also investigated the role of B cells in the pathogenesis of PBC by depleting B cells using two different monoclonal antibodies, CD20 and CD79. B cell depletion led to exacerbated cholangitis, with higher T cell infiltrates and inflammatory cytokines, indicating a protective role of B cells in PBC[107].

2OA-BSA immunized C57BL/6 mice were also studied for the potential of CTLA4-based therapy on cholangitis by using CTLA4-Ig. CTLA4-Ig is a soluble recombinant human fusion protein comprised of the extracellular domain of human CTLA4 linked to a modified portion of the Fc domain of human IgG[108,109]. In mice treated begun one day before 2-OA-BSA immunization, CTLA4-Ig completely inhibits the manifestations of cholangitis, including AMA production, intra-hepatic T cell infiltrates and bile duct damage. However, treatment with CTLA-4 Ig initiated after the development of autoimmune cholangitis in 2OA-BSA immunized mice, reduced intra-hepatic T cell infiltrates

and biliary cell damage, although AMA levels were not altered[110].

We also investigated the role of innate immunity and natural killer T (NKT) cells on modulating disease activity in this xenobiotic-induced mouse model. Briefly, we immunized mice with and without the addition of α-galactosylceramide (α-GalCer), an invariant natural killer T cell activator. 2-OA-BSA-immunized mice exposed to α-GalCer developed a profound exacerbation of their autoimmune cholangitis, including significant increases in CD8$^+$ T cell infiltrates, portal inflammation, granuloma formation, and bile duct damage. Moreover, these mice produced increased levels of AMAs and evidence of fibrosis[111]. CD4 and CD8 knock-out mice immunized with either 2-OA-BSA/PBS or 2-OA-BSA/α-GalCer develop AMAs and portal infiltrates. However, 2-OA-BSA/α-GalCer treated mice also develop fibrosis. Indeed, our data suggest that innate immunity is critical for immunopathology and that the pathology is exacerbated in the presence of α-GalCer[50]. More recently, we also reported that 2-OA-BSA-immunized mice administered with a Th2-biasing agonist (2s,3s,4r)-1-*O*-(a-D-galactopyranosyl)-*N*-tetracosanoyl-2-amino-1,3,4-nonanetriol (OCH), developed portal inflammation and hepatic fibrosis similar to mice treated with α-GalCer[75]. However, inflammatory portal cell infiltrates and AMA responses are reduced in iNKT cell deficient CD1d knockout mice treated with OCH. These results suggest that activation iNKT cells can occur *via* overlapping and/or promiscuous pathways and further highlight the role of innate immunity in the natural history of PBC.

Our data also provides clues to the mechanisms by which autoimmune diseases could be perpetuated in humans and also helps explain recurrence of PBC following liver transplantation in the absence of major histocompatibility complex (MHC) compatibility matching. Thus, in the absence of MHC restriction, disease reoccurrence would depend on a non MHC restricted cellular mechanisms, suggesting that biliary epithelial cells are simply an innocent victim of an immune attack. Thus, they attract immune attack by virtue of their unique biochemical mechanisms by which they process PDC-E2 during apoptosis[20]. Bile duct cells may have a direct effector role in immune-mediated cholangiopathies and fibrosis through their own cellular senescence pathway[112]. This also explains the suggested success of ursodiol in PBC, a drug that appears to have anti-apoptotic properties and also may modulate innate responses. Our data would also explain the relative failure of immunosuppressive drugs to alter PBC, because such agents are relatively ineffective against innate mechanisms. Finally, the induction of fibrosis in 2-OA-BSA-immunized mice exposed to α-GalCer permits not only dissection of its induction, but also has the potential to be useful in studies of intervention.
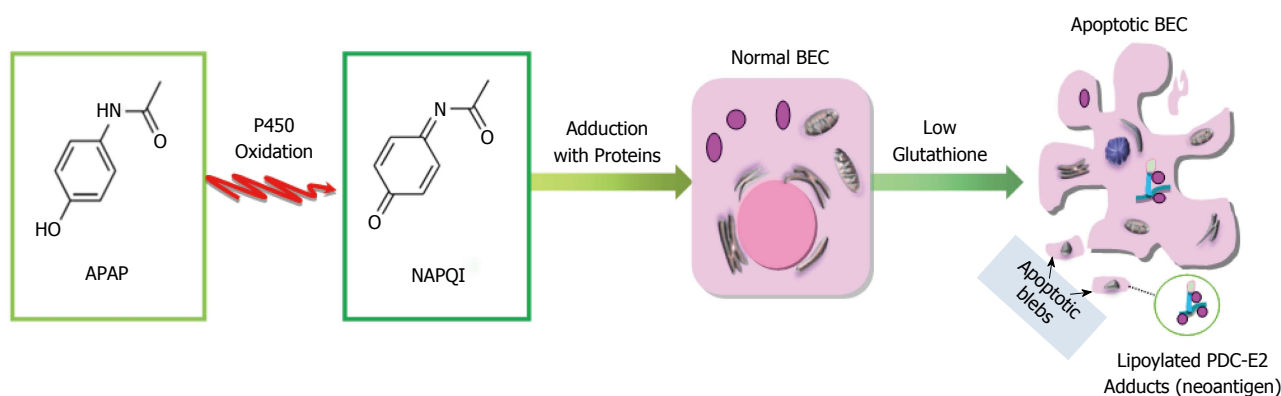
Figure 3 APAP metabolism and proposed mechanism of APAP-mediated breaking of immune tolerance. APAP is metabolized in the liver to nontoxic compounds *via* conjugation of the aromatic ring to sulfate or glucuronic acid. APAP is converted into a highly electrophilic metabolite, NAPQI by microsomal cytochrome P450 oxidation. Reactive NAPQI accumulates and can form adducts with cellular proteins, leading to disruption of cellular functions, generation of neo-antigens, and loss of tolerance.

LINK BETWEEN XENOBIOTICS AND AMA IN ACETAMINOPHEN INDUCED LIVER INJURIES

Although it is not clear how xenobiotics or the modified cellular proteins initiate autoimmunity in PBC, analysis of serum samples from subjects with acute liver failure indicate that a severe liver oxidant injury could lead to AMA production[113]. Specifically, 217 serum samples from 69 patients with acute liver failure (ALF) collected up to 24 mo post-ALF were compared with controls, for titer and reactivity with 2OADC-E2. AMA were detected in 28/69 (40.6%) ALF patients with reactivity found against all of the major mitochondrial autoantigens. The strikingly high frequency of AMAs in patients with ALF supports the thesis that oxidative stress-induced liver damage may lead to AMA induction. In particular, we note that AMA with the same antigen and epitope specificity as in patients with PBC was found in almost 35% of the acetaminophen (or APAP, chemically named N-acetyl-p-aminophenol) poisoning subjects, suggesting that the PDC-E2 lipoyl domain is likely a target of APAP induced reactive oxygen species. This finding is of significance as toxic doses of APAP produces reactive oxygen and nitrogen species and reactive metabolites[114-117] that could result in mitochondrial damage and liver injury as evidenced by the elevation of serum alanine amino transferase and P450 dependent centrilobular damage[118,119].

APAP is the most widely used non-prescription drug in the United States. Using the recommended therapeutic dosage (1000 mg per single dose and up to 4000 mg per day for adults), 85% of acetaminophen is metabolized in liver to non-toxic compounds *via* the conjugation of the aromatic ring to sulfate or glucoronic acid. The remaining 15% is converted into a highly-electrophilic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) through isozymes of microsomal cytochrome P450[120]. In the presence of the reduced form of glutathione (GSH), NAPQI can either be covalently linked to GSH *via* Michael's addition to the aromatic ring or reduced back to APAP[121]. The predominant method of NAPQI detoxification occurs through the former mechanism, resulting in depletion of the intracellular glutathione pool[122]. However, in the presence of excess APAP or when microsomal P450 is increased, hepatic GSH is depleted more extensively and cannot compete efficiently with the increased NAPQI. The resulting decrease in cellular glutathione further allows the accumulation of reactive NAPQI, which then reacts with nucleophilic sites such as cysteine and lysine residues on cellular proteins and related cofactors[123].

Previous data[124] have suggested that glutathiolation decreases the antigenicity of PDC-E2. Due to cellular depletion of glutathione, very little glutathione would be available for such covalent protection of PDC-E2. The depletion of glutathione could lead to neo-antigens through modification of native PDC-E2 by high levels of reactive NAPQI or other electrophilic agents. We reason that in PBC such electrophilic modification on lipoic-acid-conjugated PDC-E2 will inhibit the physiological function of PDC-E2 and subsequently lead to disruption of ATP synthesis, cell death and the release of either unmasked PDC-E2 or neoantigens formed by xenobiotics-modified PDC-E2. Microarray studies on APAP toxicity also revealed consistent altered transcriptome expression in oxidative phosphorylation, protein post-translational modification in liver and blood samples[125,126]. The exposure of this chemical modified self-protein to the immune system of genetically susceptible individuals could lead to the breakdown of self-tolerance to native PDC-E2 itself by molecular mimicry and epitope spreading mechanism. Thus, in genetically susceptible individuals, the prolonged exposure to electrophilic agents, such as acetaminophen may initiate and/or enhance the breakdown of self-tolerance to PDC-E2 and eventually lead to PBC (Figure 3).

CONCLUSION

The etiological mechanism of the immunological specificity of the 2-OADC-E2 enzymes lipoyl domain in PBC remains an enigma. Recent quantitative structure-activity relationship (QSAR) studies suggest that disruption of the lipoyl ring S-S linkage renders the lipoic acid "activated" and receptive for xenobiotic modification and subsequent AMA recognition[101]. Data from immunological characterization of antigen and Ig isotype specificities against one such lipoyl acid mimic SAc and rPDC-E2 strongly support a xenobiotic etiology in PBC. This observation is of significance in light of the high frequency of AMAs in patients with ALF. In particular, AMA was found in almost 35% of APAP poisoning subjects in a cohort of ALF patients[113]. Highly reactive electrophilic metabolites of APAP such as NAPQI can deplete the intracellular glutathione pool and render PDC-E2 vulnerable to further modification by electrophiles. Such mechanisms of *in vivo* generation of xenobiotic modified self proteins could lead to the breaking of tolerance to native proteins through molecular mimicry and antigen spreading in genetically susceptible individuals[102]. Finally, the recapitulation of AMA and PBC-like biliary lesions in 2OA-BSA immunized mice further support our working hypothesis on xenobiotic etiology of PBC[103]. Future work is directed at examining the biochemical and immunological mechanisms underlying the breach of tolerance in autoimmunity in PBC by environmental chemicals. Knowledge gained from this model may have significant preventive and therapeutic implications in the clinical management of PBC.

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