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Environment and Primary Biliary Cirrhosis: Electrophilic Drugs and the Induction of AMA

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Abstract

Environmental stimulation is a major factor in the initiation and perpetuation of autoimmune diseases. We have addressed this issue and focused on primary biliary cirrhosis (PBC), an autoimmune disease of the liver. Immunologically, PBC is distinguished by immune mediated destruction of the intra hepatic bile ducts and the presence of high titer antimitochondrial autoantibodies (AMA) directed against a highly specific epitope within the lipoic acid binding domain of the pyruvate dehydrogenase E2 subunit (PDC-E2). We submit that the uniqueness of AMA epitope specificity and the conformational changes of the PDC-E2 lipoyl domain during physiological acyl transfer could be the lynchpin to the etiology of PBC and postulate that chemical xenobiotics modification of the lipoyl domain of PDC-E2 is sufficient to break self-tolerance, with subsequent production of AMA in patients with PBC. Indeed, using quantitative structure activity relationship (QSAR) analysis on a peptide-xenobiotic conjugate microarray platform, we have demonstrated that when the lipoyl domain of PDC-E2 was modified with specific synthetic small molecule lipoyl mimics, the ensuing structures displayed highly specific reactivity to PBC sera, at levels often higher than the native PDC-E2 molecule. Hereby, we discuss our recent QSAR analysis data on specific AMA reactivity against a focused panel of lipoic acid mimic in which the lipoyl di-sulfide bond are modified. Furthermore, data on the immunological characterization of antigen and Ig isotype specificities against one such lipoic acid mimic; 6,8-bis(acetylthio)octanoic acid (SAC), when compared with rPDC-E2, strongly support a xenobiotic etiology in PBC. This observation is of particular significance in that approximately one third of patients who have taken excessive acetaminophen (APAP) developed AMA with same specificity as patients with PBC, suggesting that the lipoic domain are a target of APAP electrophilic metabolites such as NAPQI. We submit that in genetically susceptible hosts, electrophilic modification of lipoic acid in PDC-E2 by acetaminophen or similar drugs can facilitate loss of tolerance and lead to the development of PBC.

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Molecular mimicry and xenobiotics in autoimmune diseases

Accumulating data from epidemiological studies on autoimmunity have strongly implicated the role of environment in the etiology [1, 2]. In autoimmunity, the paradox is that autoantigens are unable to elicit a primary immune response themselves but can be recognized as targets for effector T cells stimulated by a pathogenic cross-reactive epitope. To break self-tolerance to the autoantigen, the epitope mimic or mimeotope needs to induce activation and proliferation rather than anergy of autoreactive T cells. Subsequently, the autoantigen presented by the host cells of a certain tissue must be recognized by reactive epitope-specific T cells to cause autoimmune disease. Among the hypothetical mechanisms for the environmental etiology of autoimmunity, the concept of self antigen changes induced by chemical and infectious agents which could break tolerance by post-translational modifications and molecular mimicry have received substantial attention [3]. This mechanism of molecular mimicry has been suggested to be associated with several systemic autoimmune diseases, including multiple sclerosis [4–6], systemic lupus erythematosus (SLE) [7, 8] and rheumatoid arthritis [9–11]

Although bacteria and viruses are candidates for the induction of autoimmune disease by molecular mimicry [12], there are also other environmental factors, xenobiotics or 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 [2]. Halothane hepatitis is a xenobiotics-induced liver disease that occurs when susceptible individuals develop an immune response against trifluoroacetylated (TFA) protein antigens. Exposure to TFA-conjugated self-proteins results in antibody responses against such TFA-self proteins. Interestingly, the human PBC mitochondrial autoantigen; lipoylated inner lipoyl domain of PDC-E2, but not the unlipoylated form, is also recognized by anti-TFA [13]. Here, we will first provide an overview of the natural history, genetics and immunobiology of PBC. We will also discuss the biochemistry of PDC-E2 and its potential susceptibility to xenobiotic modifications, with particular emphasis on our recent data supporting that xenobiotic modification of lipoyl-PDC-E2 and finally our hypothesis on the role of electrophilic drugs modification of PDC-E2 in breaking of tolerance in PBC.

Natural History and Genetics of Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is a liver specific autoimmune disease. The incidence of PBC is 2.7 per 100,000 in a well defined US population [14] but varies between geographic locations [15, 16]. PBC is more prevalent in Northern Europe and North America and less common in Eastern Asia, Africa, and Australia [17, 18]. Epidemiological studies suggest that the incidence of PBC is increasing [15]; it affects women predominantly with a female:male ratio of 9:1 and a middle age onset [19]. PBC is characterized by the presence of high titer antimitochondrial autoantibodies (AMA) and immune mediated progressive destruction of biliary epithelial cells (BEC) of small bile ducts, eventually leading to cholestasis, fibrosis and potentially liver cirrhosis [19]. Approximately 50–60% of patients are asymptomatic at diagnosis and the disease has a long latency period [20, 21], followed by the development of symptoms, usually fatigue, pruritus hyperpigmentation of the skin, and later bleeding varices, edema, or ascites [22]. The prognosis of patients diagnosed with PBC has improved significantly over the past 2 decades perhaps because patients are being diagnosed earlier.

The female predominance in PBC suggests there are significant genetic components in this disease, supported by the high frequency of monosomy of the X chromosome in PBC [23]. Reports from recent genetic studies demonstrate that in addition to the HLA-locus, several genetic susceptibility loci of IL12-related pathways, SPIB, IRF5-TNPO3, and 17q12-2 are

associated with PBC; new candidate genes include STAT4, DENND1B, CD80, IL7R, CXCR5, TNFRSF1A, CLEC16A, and NFKB1 have been identified by genome wide association studies [24–27]. Data on familial clustering of PBC demonstrates that first-degree relatives of PBC patients have an increased risk of developing disease. Most often, these familial clusters involve mother-daughter pairs, which is consistent with the female preponderance of the disease [28, 29]. Furthermore, twin studies have demonstrated a high concordance for PBC in monozygotic twins and a low concordance among dizygotic twins [30]. These clusters provide evidence for a genetic basis underlying PBC. On the other hand, clusters of nonrelated individuals suggest that environmental factors also play a role in the development of the disease [31]. Environmental components including chemicals [32–34] have been implicated in initiating PBC.

The understanding that environment plays a critical role in the development of autoimmunity was based in part on studies of concordance of specific autoimmune diseases in identical twins [35]. It is interesting to note that with the exception of PBC, the penetrance of autoimmunity in monozygotic twins ranges typically between 20–30%, suggesting that factors gene sequence itself is not sufficient in determining the outcome of autoimmunity [36]. Moreover, the concordance rate of multiple autoimmune diseases in monozygotic twins such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type I diabetes mellitus and psoriasis also varies considerably [3, 36, 37]. This lack of concordance in monozygotic twins in autoimmune diseases strongly suggests that environmental and/or epigenetic factors are also important in determining the susceptibility to autoimmunity. The thesis is further supported by the observation from large scale single polymorphism analysis multicenter studies and genome wide association studies that significant genetic associations between certain SNPS and autoimmunity are only limited to certain subgroups of patients [38, 39]. Compelling data from epidemiologic studies also highlighted the contribution of environmental factors in the development of human autoimmune diseases [1, 2]. The need to dissect various environmental stimuli (e.g. chemicals, toxins, drugs, pharmaceuticals, ultraviolet radiation) and their mechanisms in breaking of tolerance is eminent [2]. Given the higher incidence of autoimmunity in female [40], it will be of interest to understand how different stimuli may differentially affect males and females and the biological pathways involved [41]. Data from animal models have led to increased confidence about environmental factors that affect expression of autoimmunity [42] and constitute extremely valuable tools for studying the induction or exacerbation of autoimmunity by environmental conditions and exposures.

Immunobiology of primary biliary cirrhosis

AMA is present in over 95% of patients with PBC and is the most specific diagnostic antibody marker of PBC [43]. 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) 2-oxo-glutarate dehydrogenase complex (OGDC-E2 [44–47] and the E3 binding protein of dihydrolipoamide dehydrogenase [48]. The AMA target antigens are all localized within the inner mitochondrial matrix and catalyze the oxidative decarboxylation of 2-oxo-acid acid substrates [49]. Biochemically, 2OADC-E2 have a common functional domain containing a single or multiple lipoyl groups. The immunodominant epitopes recognized by AMA are all mapped within the lipoyl domains of these target antigens [50, 51]. In patients with PBC, T helper (CD4⁺) T cells and cytotoxic (CD8⁺) T cells are present in portal tracts, around damaged bile ducts [19]. Although PDC-E2 autoreactive CD4 T cells are present in peripheral blood and liver; there is a specific 100–150 fold increase in number of PDC-E2-specific CD4 T cells in the hilar lymph nodes and liver versus peripheral blood in patients with PBC [52]. 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 [53]. The PDC-E2 autoepitope for both CD4 and CD8 T cells, overlaps with the B cell epitope, which spans the lipoyl domain [53]. Recent reports also substantiate the involvement of innate immunity, including monocytes, toll like receptors and natural killer cells in the development of PBC [54–57]. The multi-lineage response to the PDC-E2 lipoyl domain, the immunodominant mitochondrial autoantigen in PBC, points to the thesis that loss of tolerance to PDC-E2 lipoyl domain is the initiating event that leads to the subsequent development of clinical biliary pathology [19].

PDC-E2 lipoyl domain is vulnerable to environmental xenobiotic modification

High resolution structural analysis and modeling studies of the PDC-E2 lipoyl domains from both prokaryotes and eukaryotes demonstrate 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 [58]. 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 the accessibility of its dithiolane ring for reductive acylation [59, 60] (Figure 1.) Although the change in conformation and the existence of multiple conformations of the lipoyl domain during reductive acylation is important in catalyzing the acyl transfer [59], it also renders PDC-E2 susceptible to aberrant chemical modifications. The demonstration of antibodies directed against free lipoic acid and a carrier-conjugated form of lipoic acid are present in AMA positive sera [61] has prompted us to rationalize that conjugated form(s) of native or xenobiotic lipoic acid mimics could contribute to the initiation and perpetuation of autoimmunity by at first breaking self-tolerance and participating in subsequent determinant spreading. Indeed, using QSAR analysis on a microarray platform, we demonstrated that when the lipoyl domain of a PDC-E2 peptide was conjugated to a number of synthetic small molecule lipoyl mimics, a number of the ensuing structures displayed highly specific reactivity to AMA positive PBC sera, at levels often higher than the native PDC-E2 molecule [33, 62, 63]. Interestingly, these chemicals include compounds that are widely used in the environment including perfumes, lipstick, and many common food flavorings [33, 63].

Alteration of the PDC-E2 lipoyl ring enhances AMA binding

To address whether direct alteration of the lipoyl ring – i.e., disruption of the S-S linkage – renders the lipoic acid “activated” and receptive for xenobiotic modification and subsequent AMA recognition [62], we have performed QSAR analysis on a focused panel of lipoic acid mimic in which the lipoyl di-sulfide bond are modified. Our results demonstrated that 6,8-bis(acetylthio)octanoic acid (SAC), 8-(acetylthio)octanoic acid (OASAc), and 6,8-bis(propionylthio)octanoic acid (SCOEt) were highly reactive to sera from AMA-positive PBC patients, at levels even higher than the reactivity against lipoic-acid-conjugated PDC-E2 peptide (Table 1). Structural analysis of these three compounds suggests that they are in fact quite similar to what happens naturally during physiological reactions. These thioester modified analogs are known to arise from acylation of reduced lipoic acid. That is, these compounds contain a reduced lipoic acid with the addition of one (OASAc) or two (SAC) acetyl groups or the addition of two propionyl groups (SCOEt). Interestingly, the oxidative states of lipoic acid have been shown to affect the immunogenicity of PDC-E2 [64]. The lipoic-acid-conjugated PDC-E2 is more immunogenic if it is in a reduced form [65], which corresponds to the ring-open form of lipoic acid. Altogether, these findings suggest that

xenobiotic modification of the lipoyl S-S linkage of a physiological metabolite during lipoyl electron transfer could break tolerance to PDC-E2 and elicit AMA.

To further determine the hapten specificities to SAc in patients with PBC, we performed inhibition ELISA and also prepared affinity-purified antibodies against rPDC-E2, SAc-BSA, and SAc-RSA conjugates. Interestingly, affinity purified antibodies against rPDC-E2 bound to only rPDC-E2 and not to SAc-BSA or SAc-RSA. In contrast, SAc-conjugate affinity purified antibodies reacted to both SAc-conjugates and rPDC-E2. Furthermore, we performed isotyping on the affinity purified antibodies to determine the major Ig classes. The affinity purified SAc-conjugate reactive antibodies and the affinity purified rPDC-E2 reactive antibodies displayed IgG reactivities against rPDC-E2. Little to no SAc-conjugate-purified and rPDC-E2-purified antibodies were detected against SAc-conjugated proteins using anti-IgG secondary antibodies. SAc-conjugate-purified antibodies demonstrated IgM reactivities against both rPDC-E2 and SAc-conjugates. However, when SAc-conjugate-purified antibodies were tested against rPDC-E2 the binding population was found to be predominantly IgG ($p < 0.0001$) (Figure 2). Collectively, our data on the specificities and Ig isotype analysis against xenobiotic-PDC-E2 in PBC sera suggest that initial exposure to chemicals such as xenobiotic-modified PDC-E2 leads to a primary IgM specific immune response against the antigen, e.g. SAc. Subsequently, the similarity between the lipoyl domain of PDC-E2 and the xenobiotic-modified lipoyl domain of PDC-E2 (SAc-moiety) generates cross-reactive immune responses against the self-antigen, leading to the self-tolerance breakdown to the lipoyl domain of mitochondrial PDC-E2. Later, through the process of affinity maturation and isotype switching, the secondary immune response produces IgGs that are highly specific for mitochondrial PDC-E2 [66].

Clues for a link between xenobiotic and AMA in acetaminophen induced liver injuries

The human liver is at risk for drug induced injury because many environmental chemicals are metabolized primarily by hepatocytes [67]. The pathogenesis of most drug-induced liver injuries is initiated by the metabolic conversion of xenobiotics into reactive intermediate species, such as electrophilic compounds or free radicals, which can readily alter the structure and function of cellular macromolecules to form neo-antigens. Furthermore, reactive intermediate species could lead to oxidative stress, deregulation of cell signaling pathways, dysfunction of biomolecules, organelles malfunction and eventual cell death [68]. 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 severe liver oxidant injury could lead to AMA production [69]. Specifically, 217 serum samples from 69 patients with acute liver failure (ALF) collected up to 24 months 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 against all of the major mitochondrial autoantigens. This observation 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 (APAP) 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 [70, 71] that could result in mitochondrial damage and liver injury as evidenced by the elevation of serum alanine amino transferase and P450 dependent centrilobular damage [72, 73].

Mechanism of APAP metabolism and protein modification

APAP is the most widely used non-prescription drug in the United States. Using the recommended therapeutic dosage of (1,000 mg per single dose and up to 4,000 mg per day for adults), 85% of the acetaminophen is metabolized in liver to non-toxic compounds via the conjugation of the aromatic ring to sulfate or glucuronic acid. The remaining 15% is converted into a highly-electrophilic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) through isozymes of microsomal cytochrome P450 [74]. 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 [75]. The predominant method of NAPQI detoxification occurs through the former mechanism, resulting in depletion of the intracellular glutathione pool [76]. 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 react with nucleophilic sites such as cysteine and lysine residues on cellular proteins and related cofactors [77].

Previous data [49] have suggested that glutathiolation would decrease the antigenicity of PDC-E2. However, due to cellular depletion of glutathione under excessive APAP, very little glutathione would be available for such covalent protection of PDC-E2. The depletion of glutathione could lead to possible 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. 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 (Figure 3). 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.

Significance of AMA in the pathophysiology of BEC in PBC

Despite the fact that the mitochondrial targets are ubiquitous proteins expressed in all nucleated cells, the immunopathology of PBC is characterized by the presence of CD4⁺ and CD8⁺ T cell infiltrates and the specific destruction of BEC [19]. This apparent paradox suggests there are unique immunopathological characteristics in BEC, the target tissue. Previous work suggested that AMA preferentially recognize PDC-E2 with reduced sulfhydryl groups and hence blocking these sites would likely eliminate the epitope site responsible for the generation of autoantibodies [65]. This concept was further supported by studies directed at characterizing the autoantibody recognition site and the effects of apoptosis on the immunogenicity of PDC-E2 [64]. The authors demonstrated that, after apoptosis, HeLa, Jurkat T, and Caco-2 cells presented a form of PDC-E2 that becomes undetectable by AMA. This loss of recognition of apoptotic cell-derived PDC-E2 by AMA is not attributable to disappearance or degradation, but rather to a reversible structural change in the protein from which the epitope can be recovered. In contrast, AMA recognition of PDC-E2 could be detected in apoptotic BEC. This immunoreactivity can be eliminated with the addition of oxidized glutathione to sodium dodecyl sulfate-treated cholangiocyte cell lysates that render PDC-E2 nonantigenic when probed with AMA. Conversely, apoptotic HeLa cells pretreated with buthionine sulfoximine (a glutathione synthetase inhibitor) before apoptotic stimulus were unable to prevent the recognition by patient sera, thereby "resurrecting" the antigen with depletion of glutathione. The authors

concluded that the persistent recognition of PDC-E2 derived from cholangiocytes is caused by a failure in these cells to covalently link PDC-E2 to glutathione during the course of apoptosis. The depletion of GSH by NAPQI may be important in the outcome of immunogenicity of PDC-E2 in the context of APAP toxicity

Apoptosis of BEC has been proposed as a potential source of neo-antigens that are responsible for activating autoreactive lymphocytes [78]. It is also important to note when BECs, but not control cells, undergo apoptosis, PDC-E2 remains immunologically intact [64, 79]. More importantly, when monocyte-derived macrophages from PBC patients were incubated with apoptotic bodies from BECs in the presence of AMAs, there was intense pro-inflammatory cytokine production. This production was inhibited by anti-CD16, an antibody against the Fc receptor [80]. In light of the fact that AMA can be induced in patients with APAP overdose, these recent findings on apoptotic BECs further emphasize a unique role of AMAs in the etiopathogenesis of PBC.

Conclusions

The etiological mechanism of the immunological specificity of the 2-OADC-E2 enzymes lipoyl domain in PBC remains an enigma. We now have evidence from QSAR studies when the lipoyl domain of PDC-E2 was modified with specific synthetic small molecule lipoyl mimics, the ensuing structures displayed very specific reactivity to PBC sera, at levels often higher than the native PDC-E2 molecule. We believe that the ability of lipoic acid to rotate by means of its “swinging arms” with respect to the bulk of the entire PDC-E2 molecule makes the dithiolane ring vulnerable to xenobiotic modification. Recent QSAR analysis a focused panel of lipoic acid mimic in which the lipoyl di-sulfide bond are modified, suggest that direct alteration of the lipoyl ring – i.e., disruption of the S-S linkage – renders the lipoic acid “activated” and receptive for xenobiotic modification and subsequent AMA recognition [62]. 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, we note that AMA with the same antigen and epitope specificity as in patients with PBC, was found in almost 35% of APAP poisoning subjects, suggesting that the PDC-E2 lipoyl domain is likely a target of APAP induced reactive oxygen species. The generation of highly reactive electrophilic metabolites such as NAPQI, which also deplete the intracellular glutathione pool could 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.

List of Abbreviations

AMA	antimitochondrial autoantibodies
APAP	acetaminophen
BCOADC-E2	E2 subunits of branched chain 2-oxo acid dehydrogenase complex
OADC-E2	E2 subunits of the 2-oxo-acid dehydrogenase complexes
OGDC-E2	E2 subunits of 2-oxo-glutarate dehydrogenase complex
OASAc	8-(acetylthio)octanoic acid
PBC	primary biliary cirrhosis
PDC-E2	E2 subunits of pyruvate dehydrogenase

QSAR	quantitative structure activity relationship
NAPQI	N-acetyl-p-benzoquinoneimine
SAc	6,8-bis(acetylthio)octanoic acid
SCOEt	6,8-bis(propionylthio)octanoic acid
TFA	trifluoroacetylated

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1. AMA is a powerful probe in identifying chemical environmental footprints that are important in the etiology of PBC.
2. A number of chemical mimics of lipoic acid which recognize AMA better than the native PDC-E2 peptide were identified using QSAR analysis on a peptide-xenobiotic conjugate microarray platform. These chemical mimics include compounds that the disulfide bonds of lipoic acid are disrupted.
3. Data on the immunological characterization of antigen and Ig isotype specificities against one such lipoic acid mimic; 6,8-bis(acetylthio)octanoic acid (SAC), when compared with rPDC-E2, strongly support a xenobiotic etiology in PBC.
4. We noted that one third of patients with acute liver failure who have taken excessive APAP developed AMA with same antigen specificity as in patients with PBC; suggesting that the lipoic domain are likely a target of APAP electrophilic metabolites such as NAPQI.
5. In genetically susceptible hosts, electrophilic modification of lipoic acid in PDC-E2 by APAP or similar drugs can lead to the loss of tolerance to PDC-E2 and the development of PBC.

Figure 1.

Diagrammatic representation of the swinging arms of lipoic acid on PDC-E2. Note that lipoic acid is covalently attached to the ϵ group of lysine via an amide bond and is prominently displayed on the outer surface of PDC-E2, which allows the accessibility of the lipoyl dithiolane ring for reductive acylation.

Figure 2.
Schematic representation of reactivity patterns of SAc reactive and rPDC-E2 reactivity antibodies and their isotypes in AMA positive PBC sera.

Figure 3. APAP metabolism and hypothetical mechanism of APAP metabolite modification of PDC-E2; AMA generation via neoantigen and molecular mimicry and subsequent immune mediated destruction of bile duct epithelial cells in PBC.

Microarray analysis of IgM and IgG reactivity of AMA positive PBC patients and healthy controls against lipoic acid conjugated-PDC-E2 peptide and a focused panel of xenobiotics-modified PDC-E2 peptide.

Table 1

Xenobiotics	Structure	IgM		IgG	
		AMA-positive PBC	Healthy control	AMA-positive PBC	Healthy control
LA		3544 ±508.4 ***	130.5 ±74.03	1364 ±198.6 ***	242.4 ±57.81
MA		-26.00 ±50.35	-114.6 ±62.44	-92.39 ±35.85	-47.68 ±73.35
2OA		3730 ±482.8 ***	149.9 ±96.47	512.1 ±157.7 **	-83.68 ±125.0
OASAc		12860 ±1793 ***	694.7 ±189.9	842.9 ±196.1 ***	-5.821 ±125.3
SFn		615.5 ±245.0 **	-102.1 ±66.58	-99.24 ±55.75	-275.1 ±107.4
SAC		12480 ±1798 ***	1763 ±333.8	2756 ±640.2 ***	158.8 ±235.0

Xenobiotics	Structure	IgM		IgG	
		AMA-positive PBC	Healthy control	AMA-positive PBC	Healthy control
SCOEt		10040 ± 1491 ***	1587 ± 475.2	1130 ± 265.7 ***	-155.2 ± 109.0
SCOPh		4625 ± 775.9 ***	209.8 ± 105.0	304.7 ± 93.45 **	-101.4 ± 119.4
SMe		380.9 ± 153.1	257.8 ± 138.1	-513.7 ± 99.59	-718.0 ± 168.0
SOMe		304.9 ± 224.1	576.9 ± 238.6	-686.1 ± 135.3	-1008 ± 237.2
SO ₂ Me		-340.6 ± 69.77 **	-37.29 ± 64.33	-509.5 ± 119.3	-220.0 ± 179.9

Asterisks indicate significant differences (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; two-tailed unpaired t -test with Welch's correction).

LA: lipoic acid, MA: methacrylic acid, 2OA: 2-octynoic acid, OASAc: 8 (acetylthio)octanoic acid, SFm: 6,8-bis(formylthio)octanoic acid, SAc: 6,8-bis(acetylthio)octanoic acid, SCOEt: 6,8-bis(propionylthio)octanoic acid, SCOPh: 6,8-bis(benzoylthio)octanoic acid, SMe: 6,8-bis(methylthio)octanoic acid, SOMe: 6,8-bis(methylsulfonylthio)octanoic acid, SO₂Me: 6,8-bis(methylsulfonylthio)octanoic acid.