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Immunoreactivity of Organic Mimeotopes of the E2 Component of Pyruvate Dehydrogenase: Connecting Xenobiotics with Primary Biliary Cirrhosis

S. Alice Long,* Chao Quan,‡ Judy Van de Water,* Michael H. Nantz,‡ Mark J. Kurth,‡ Daniel Barsky,§ Michael E. Colvin,§ Kit S. Lam,† Ross L. Coppel,‖ Aftab Ansari,‖ and M. Eric Gershwin2*

In primary biliary cirrhosis (PBC), the major autoepitope recognized by both T and B cells is the inner lipoyl domain of the E2 component of pyruvate dehydrogenase. To address the hypothesis that PBC is induced by xenobiotic exposure, we took advantage of ab initio quantum chemistry and synthesized the inner lipoyl domain of E2 component of pyruvate dehydrogenase, replacing the lipoyc acid moiety with synthetic structures designed to mimic a xenobiologically modified lipoyl hapten, and we quantitated the reactivity of these structures with sera from PBC patients. Interestingly, antimitochondrial Abs from all seropositive patients with PBC, but no controls, reacted against 3 of the 18 organic modified autoepitopes significantly better than to the native domain. By structural analysis, the features that correlated with autoantibody binding included synthetic domain peptides with a halide or methyl halide in the meta or para position containing no strong hydrogen bond accepting groups on the phenyl ring of the lysine substituents, and synthetic domain peptides with a relatively low rotation barrier about the linkage bond. Many chemicals including pharmaceuticals and household detergents have the potential to form such halogenated derivatives as metabolites. These data reflect the first time that an organic compound has been shown to serve as a mimeotope for an autoantigen and further provide evidence for a potential mechanism by which environmental organic compounds may cause PBC. The Journal of Immunology, 2001, 167: 2956–2963.

Primary biliary cirrhosis (PBC), a disease found predominantly in females, leads to the destruction of intrabiliary bile ducts. Although the etiology of PBC is unknown, there are several clues. First, it is never found in childhood. Second, it is more common in Westernized countries, and the incidence may be increasing (1). Third, despite the absence of association with the MHC, the risk factor for developing PBC in a first-degree relative is 100- to 800-fold more common, and the onset of disease in the relative is often within a few years of the other’s diagnosis (2). Fourth, there is a long incubation time between the appearance of antimitochondrial Abs (AMAs) and clinical disease. Fifth, AMAs, detected by using recombinant autoantigens as targets, are pathognomonic of PBC. Sixth, there are no objective data supporting a role for microbial agents in the etiology of PBC (3).

Xenobiotics are foreign compounds that may either alter or complex to defined self proteins, inducing a change in the molecular structure of the native protein sufficient to induce an immune response. Such immune responses may then result in the recognition of not only the modified or altered protein, but also the unmodified native protein (4, 5). The chronic presence of the self protein serves to perpetuate the immune response initiated by the xenobiologically induced adduct and leads to autoimmunity (6, 7). Many xenobiotics are metabolized in the liver, thereby increasing the potential for liver-specific alteration of proteins (8). In fact, a liver-specific autoimmune disease can be observed in some patients exposed to chlorofluorohydrocarbon anesthetics (9, 10). Previous work has reported that immunization with halothane, whose trifluoroacetyl (TFA) metabolite covalently links to lysine on cytochrome p450 2E1 (11), induces the formation of Abs that cross-react not only with the halogenated (TFA) immunogen, but also to the lipoylated E2 component of pyruvate hydrogenase (PDC-E2), the major autoantigen of PBC (12, 13). This finding has important implications in the pathogenic mechanisms associated with PBC, an autoimmune disease marked by the presence of AMAs (14, 15). The target of AMAs are the E2 components of the 2-oxo acid dehydrogenase pathway, particularly PDC-E2 (14), and the primary B cell epitope of PDC-E2 recognized by AMAs includes a lipoylated lysine residue (16, 17).

We hypothesize that the lipoyl acid residue of PDC-E2 serves as a xenobiotic target that, following the modification of the lipoyl lysine residue, becomes immunogenic and initiates or perpetuates an AMA response. We further hypothesize that the AMA response is induced by a modified self protein and that the Ab specificities present in such sera include those that recognize the xenobiologic modification. Herein, we took advantage of a microbead system for which peptide synthesis, derivatization, and determination of
Ab reactivity can all be performed on the same solid support (18). Our data reflect, for the first time, that organic chemicals, some of which could occur after exposure to common environmental agents, can serve as a more effective epitopes than the native autoantigen. These results may have significant implication for the pathogenesis of PBC.

Materials and Methods

Microbead ELISA

Approximately 500 beads with the appropriate peptide organic mimeotope bound via a polyethylene glycol linker were incubated in a polypropylene column (PerkinElmer Wallac, Gaithersburg, MD) containing 90 μg TentaGel resin at 0.27 mmol/g (Rapp Polymere, Tübingen, Germany) as previously described (18). Each bead has 100 pmol peptide conjugate (19). Columns containing beads were first blocked with 1% BSA in PBS for 30 min. The 1% BSA was removed, and the beads were incubated with 300–500 μl of sera diluted 1/1000 at room temperature for 1–3 h. After incubation with sera, the columns were washed four times with PBS and 1% Tween 20. Ab binding was quantitated by the addition of HRP-labeled secondary anti-human IgG, IgA, and IgM Abs diluted 1/2000 (BioSource International, Camarillo, CA). After 30 min, the beads were washed, ABTS substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) added, and the OD read at 405 nm.

Affinity purification of sera

Briefly, 100 μg purified recombinant human PDC-E2 (20) was separated on a 10% SDS-PAGE and transferred to nitrocellulose. The separated recombinant protein was cut from the nitrocellulose and blocked with 1% milk for 30 min before incubation with sera diluted 1/5. The strips were extensively washed and then subjected to elution with 0.1 M glycine-HCl, 20 mM NaCl, 0.1 M KCl at pH 2. The eluate was immediately extensively washed and then subjected to elution with 0.1 M glycine-HCl, pH 10 polypropylene column (PerkinElmer Wallac). The resin was washed with anhydrous pentane three times under nitrogen. Next, the 18 compounds included NHS esters from acid chloride (compounds 4 and 8). Pyridine (0.24 mL, 3.0 mmol) and NHS (288 mg, 2.5 mmol) were dissolved in 10 ml CH2Cl2, placed under a nitrogen atmosphere, and treated as described above. The solution was washed with 10% HCl, saturated NaHCO3, water, saturated NaCl, and once again with water. The organic phase was dried, evaporated, and purified by recrystallization from isopropanol. Compound purity was verified by recrystallization from CDCl3 (95% yield) was obtained by evaporating the solvent (18). H NMR (CDCl3, 62.67–2.74 (t, 2H, CH3), 2.99–3.04 (t, 2H, CH2), 7.40–7.41 (d, 3H, CH), 7.47 (s, 3H, CH).

Computational methods

All lysine substituents shown in Fig. 1, except for compound 6, were optimized by ab initio quantum chemical methods, with a hydrogen atom substituted for the lysine side chain. The optimizations were done in the gas phase using a density functional method (B3LYP) and a 6–31+G(dp) basis set (23, 24). Solvent energy calculations were performed on the optimized structures using the conductor-like solvent model (25) at the B3LYP/6–31+G(d,p) level and the Gaussian 98 program (26). Compound 6 was not optimized due to the presence of iodine, which cannot be accurately modeled without including relativistic corrections.

Results

A lipoated peptide of at least 12 residues is required for AMA activity

First, to verify the epitope most commonly shared by PBC patients, sera from 10 patients were assayed against select peptides within the inner lipoyl domain of PDC-E2 (17, 21, 22). Because the vast majority of PBC patients possess AMAs that react with a linear epitope, conformational epitopes were not addressed in this study (14). We prepared varying lengths (7-, 9-, 10-, and 12-mers) of peptides encompassing the previously defined inner lipoyl domain of PDC-E2. These included lipoated and nonlipoated 7-residue amino acid 171–177 (ETDKATI), 9-residue amino acid 170–178 (IETDKATIG), 10-residue amino acid 170–179 (IETDKATIGE), and 12-residue amino acid 173–184 (DKATIGFEVQEE) peptides. We chose the N terminus acylated, lipoated 12-mer 173–184 because, in an ELISA, the mean OD of binding in sera from 10 patients with PBC was highest against this peptide and >3 SD above the mean of the control group (data not shown).

Subsequently, we used this 12-mer as the representative PDC-E2 autoepitope to analyze PBC patient sera affinity purified against full-length PDC-E2 (27). Representative affinity-purified sera from 10 PBC patients and 10 healthy control individuals were tested by ELISA for reactivity against nonlipoated and lipoated forms of the 12-mer PDC-E2 peptide. The OD for PBC sera against the lipoated native peptide was 0.49 ± 0.07 compared with 0.1 ± 0.04 for the nonlipoated native peptide (Fig. 1). OD values of the control sera were all in the background range. The data clearly indicate that lipoic acid is necessary for binding of the AMA to this peptide. Because the dominant epitopic region of PDC-E2 that encompasses both the T and B cell epitopes includes the 12-mer, we reasoned that the lipoated native peptide has the highest potential to serve as a target for modification by a xenobiotic agent.

Select halogenated conjugates create mimeotopes

To determine whether there is evidence for a xenobiocid-induced modification of this region of PDC-E2, we replaced the lipoated lysine on the PDC-E2 peptide with one of 18 lysine modifications (K+) (Fig. 1). These compounds were selected to offer a range of steric features and functional groups, with many of them designed to act as a cyclic scaffold for a halogenated compound. The compounds can be divided into four groups: group I includes rigid, short structures; group II includes linear, flexible structures; group III includes rigid, extended aromatic compounds; and group IV includes flexible, conformationally flexible structures.
Figure 1. The immunological activity and structural configuration of the PDC-E2 mimeotopes. *PBC sera against free amine peptide differs significantly from lipoped peptide (p < 0.01). †, The OD was determined by ELISA using the PDC-E2 peptide plus the chemical substituent. ‡, Ab initio quantum-chemical geometry optimizations were performed on substituents as shown but with the peptide complex replaced by hydrogen (Z=H). To give a measure of the length of the molecules, N-X distances are defined as the distance between the amidal nitrogen and the farthest halogen; N-A distances are between the amidal nitrogen and the farthest surface of the most distant atom. NA, Not applicable.

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Chemical Structure</th>
<th>O.D. 405 nm</th>
</tr>
</thead>
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<tr>
<td>Non-lipoated native peptide*</td>
<td>Ac-DKATIGPEFQEE--amide</td>
<td>0.1±0.04</td>
</tr>
<tr>
<td>Lipoped native peptide</td>
<td></td>
<td>0.49±0.07 NA/10.3</td>
</tr>
<tr>
<td>1/1</td>
<td>O.NH</td>
<td>0.08±0.04 5.4/6.4</td>
</tr>
<tr>
<td>1/2</td>
<td>O.NH</td>
<td>0.1±0.04 7.2/7.9</td>
</tr>
<tr>
<td>1/3</td>
<td>O.NH</td>
<td>0.51±0.05 7.4/8.1</td>
</tr>
<tr>
<td>1/4</td>
<td>O.NH</td>
<td>0.17±0.04 7.0/7.7</td>
</tr>
<tr>
<td>1/5</td>
<td>O.NH</td>
<td>0.27±0.09 7.08/1</td>
</tr>
<tr>
<td>1/6</td>
<td>O.NH</td>
<td>0.27±0.04 Not optimized</td>
</tr>
<tr>
<td>1/7</td>
<td>O.NH</td>
<td>0.52-0.04 8.09/1</td>
</tr>
<tr>
<td>1/8</td>
<td>O.NH</td>
<td>0.57±0.06 7.78/7</td>
</tr>
<tr>
<td>1/9</td>
<td>O.NH</td>
<td>0.28±0.05 3.04/0</td>
</tr>
<tr>
<td>1/10</td>
<td>O.NH</td>
<td>0.3±0.08 NA/11.3</td>
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</tbody>
</table>
includes an example of a flexible, extended aromatic compound. We reasoned that structures resembling the initiating xenobiologically modified epitope would bind autoantibodies with a higher affinity than lipoic acid. The K^M peptides were assayed by the microbead ELISA using PBC and control sera. Data are represented as experimental values subtracted from background data (i.e., data from control sera) for each compound tested. A variety of binding strengths were observed (Fig. 1). Of interest is that peptides 13, 14, 15, and 18 showed significantly higher binding with aliquots of the same PBC sera as compared with reactivity against the same amount of the native protein. This result fits our hypothesis that these modified peptides would be more similar to the xenobiologically modified substrate. Overall, the group I benzamides have the lowest binding, the group II alkanamides are more reactive, and the groups III and IV cinnamides are the most reactive.

Structure function relationships
The structural features of the peptide lysine substituents (the “M” groups attached to the lysine side chain of the K^M peptides) were further analyzed by ab initio quantum chemistry. Within group III, there is little chemical difference between the meta- and para-trifluoromethyl substituents or between the para-trifluorotrifluoromethylated and para-chlorinated substituents, and the immunological activities of the corresponding K^M peptides are all similar. However, two K^M peptides exhibit considerably less activity, one with an ortho-trifluoromethylated substituent, K^M peptide 13, and the other with a meta-methylated substituent, K^M peptide 16. This contrast could arise from two interrelated effects: the steric fit of the K^M peptide within the binding site and the binding free energy thereof. The former depends primarily on the size and flexibility of the compound, the latter on the electrostatic and solvation properties. Although calculated solvation energies of the substituents failed to correspond to the OD, the distance measured between the amidal nitrogen atom and the farthest halogen correlates directly, if weakly, with binding activity, as measured by OD (Fig. 1, N-X; R^2 = 0.61, excluding nonhalogenated compounds). However, when we considered the distance from N to the farthest atomic surface of all the molecules, we found no correlation, except perhaps a minimum size criterion (Fig. 1, N-A). Putting all
the data together, we can propose a qualitative list of features that correlate with high binding affinity to the Ab pocket: the pocket favors $K^M$ peptides with a halide or methyl halide in the meta or para position on the phenyl ring of the lysine substituent, $K^M$ peptides that do not contain highly polar groups on the phenyl ring of the lysine substituents, and $K^M$ peptides with a relatively low barrier to rotation about a linkage bond in the lysine substituent.

**AMAs require the peptide backbone of the native modified proteins**

Other compounds are of interest because of their unique relationship to lipoic acid. Interestingly, octanoic acid (10, group II), a precursor of lipoic acid, fails to react well with AMA. In addition, Abs reactive against malondialdehyde, a byproduct of arachidonic acid metabolism, and 4-hydroxy-2-nonenal also failed to react with $K^M$ peptides (data not shown) (28–30).

To ascertain features of the molecule that may be involved in tolerance breakdown, we investigated whether a high degree of reactivity requires the specific peptide sequence of the inner lipoyl domain of PDC-E2 (15). Six representative sera were affinity purified against native lipoated PDC-E2 and screened for reactivity (Fig. 2). Altered peptides included peptide 1 with a D173A mutation, peptide 2 with a double K174E and E183K mutation, and an albumin peptide. AMA failed to react with peptides in which the amino acids flanking the lysine residue were altered, moved, or replaced by an irrelevant amino acid/residue. Thus, in addition to the chemical substituents, the native PDC-E2 peptide backbone is required for reactivity.

**Heterogeneous responses of individual patients**

Collectively, AMAs of multiple PBC patients cross-reacted with both the native lipoated peptide and select $K^M$ peptides. However, individual patients have a heterogeneous population of AMAs that react with multiple $K^M$ peptides, as shown in Table 1 by the responses of aliquots of a single serum from individual patients. For example, patient 1 responded to all four of the selected peptides although at different levels. Moreover, the pattern of reactivity is relatively different for each individual patient as seen by comparing patients 6 and 8. In addition, reactivity of each compound produces varied levels of AMA as shown by AMA dilutions for each patient, which fail to correspond to the overall level of reactivity for each individual patient tested.

**Hypothesized pathway for the addition of halogenated compounds**

In light of the data shown above, we propose that $K^M$ peptides may not only effectively mimic lipoic acid in vitro, but that exposure to environmental organics similar to halothane may also lead to modification in vivo of lipoic acid (Fig. 3). To test this theory, we synthesized one of the possible products of halothane metabolism, trifluoroacetalddehyde, and coupled this with the reduced bi-mercaptan form of lipoic acid to give R/S-5-(2-trifluoromethyl)-1,3di-thian-4-yl)pentanoic acid. We found the $K^M$ peptide of this compound to be highly AMA reactive (Fig. 4), meaning this compound, with a trifluoromethyl group, fits the quantitative structure-activity relationships profile of long, flexible xenobiologically halogenated agent. Therefore, it supports our etiological hypothesis that patient AMAs showed a greater affinity to the $K^M$ peptide than lipoic acid and an even greater reactivity than that of all $K^M$ peptides tested previously (refer to Fig. 3).

**Discussion**

Our laboratory has had a long-standing interest in defining the etiological basis of PBC (14). Thus, although we and others have identified the major autoantigens of PBC, it is not clear as to the event(s) that initiate(s) the recognition of these self-proteins as immunogenic. One of our working hypotheses has been that potential modifications of self proteins by agents such as xenobiotics may alter these self proteins enough to cause a breakdown of tolerance (i.e., immune responses that cross-react with mature self molecules). In this way, the immune response would be perpetuated by the chronic low-level turnover of the self protein. The autoantigen PDC-E2 is essential for the function of mitochondria by using lipoic acid to rapidly transition between an oxidized and reduced state. Moreover, porcine PDC-E2 loses antigenicity when lipoic acid is removed (31), confirming our observation that lipoic acid is necessary for reactivity. Perhaps the preference for enzymes containing substituents like lipoic acid can be explained because the functional site of the enzymes are more accessible and therefore more susceptible to modification by exogenous agents (e.g., xenobiotics), thus providing neoantigens that can be recognized by the immune system (32).

Several interesting elements were revealed in this study, including our observation that affinity purified autoantisera reacted with multiple modified organic structures. The fact that reactivity was significantly greater than that of the native peptide in several cases (groups III and IV) raises two important points. More than one form of a xenobiotic may be able to generate an autoantigenic determinant that would culminate in the production of anti-PDC-E2 Abs. Also, these results provide strong evidence for a structural requirement of PDC-E2 recognition with respect to the lysine-bound side-chain moiety.

The computational studies have revealed properties that both correlate with activity, namely size and flexibility (see below), and that do not correlate with activity, most notably hydrophobicity. In...
the absence of structural and biochemical observation of the interactions between the substituent, the peptide and the Ab, the structure vs activity relationships of the substituents can provide important clues toward those interactions. By quantum chemical calculations, we have pursued the idea that the rather surprising difference in binding between the ortho-trifluoromethyl-substituted K<sup>M</sup> peptide 13 and the meta-methyl substituted K<sup>M</sup> peptide 16—an apparent dependence on halogens for xenobiotic influence—could be due to an electron-withdrawing effect of the halogen in the lysine substituent of K<sup>M</sup> peptide 13 that allows greater torsional freedom in the C-C single bond linking the phenyl ring to the alkene by reducing the degree of resonance-induced double-bond character in this bond. This idea is reinforced by quantum chemical geometry optimization of the lysine substituent of the most highly active K<sup>M</sup> peptide 18, where the linkage contains no double bond and where, unlike all of the alkenes, the ring of the lysine substituent optimized to an orientation perpendicular to the amidal group. Thus, we calculated the torsional barriers of the C-C bond nearest the ring; in solvent, the barrier was indeed lowered slightly by the presence of the halogens, by 0.5 (substituent on K<sup>M</sup> peptide 16) kcal/mol to halothane, that Kupffer cells carry tri...determinant lipoyl domain has provided strong evidence that xenobiotic modification could be the initiating event of PBC.

The liver is an important organ for metabolism/degradation for xenobiotics and an altered immune response (34). A large number of chemicals, including halogenated compounds, are detoxified through the liver and secreted in the bile. Hence, exposure to an agent that would uniquely modify the mitochondrial Ags within biliary epithelium could lead to a breakdown of tolerance and induction of a self-reactive response that is target specific. Moreover, there is evidence based on in vivo studies in guinea pigs exposed to halothane, that Kupffer cells carry trifluoracetylated protein adducts (35); these protein adducts are not found in other organs, including hilar lymph nodes. This provides evidence that the generation of autoactivity to the protein adducts is likely a local liver response. We should also note the possibility that xenobiotics have

<table>
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<tr>
<th>PBC Patient No.</th>
<th>Lipoated Peptide</th>
<th>II/9</th>
<th>II/11</th>
<th>III/13</th>
<th>III/16</th>
<th>AMA&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1</td>
<td>0.67 ± 0.01</td>
<td>0.91 ± 0.06</td>
<td>0.78 ± 0.06</td>
<td>1.08 ± 0.07</td>
<td>0.98 ± 0.05</td>
<td>1:2000</td>
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<tr>
<td>2</td>
<td>0.60 ± 0.07</td>
<td>0.89 ± 0.08</td>
<td>0.61 ± 0.09</td>
<td>0.92 ± 0.03</td>
<td>0.88 ± 0.03</td>
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</tr>
<tr>
<td>3</td>
<td>0.45 ± 0.09</td>
<td>0.64 ± 0.05</td>
<td>0.43 ± 0.12</td>
<td>0.74 ± 0.10</td>
<td>0.53 ± 0.10</td>
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<td>4</td>
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<td>0.69 ± 0.08</td>
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<tr>
<td>5</td>
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<td>0.88 ± 0.04</td>
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<td>1:2000</td>
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<tr>
<td>6</td>
<td>0.55 ± 0.07</td>
<td>0.66 ± 0.07</td>
<td>0.56 ± 0.03</td>
<td>0.61 ± 0.03</td>
<td>0.87 ± 0.04</td>
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<tr>
<td>7</td>
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<td>0.76 ± 0.07</td>
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<tr>
<td>8</td>
<td>0.45 ± 0.05</td>
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<td>0.56 ± 0.03</td>
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<td>0.44 ± 0.06</td>
<td>1:1000</td>
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<tr>
<td>9</td>
<td>0.66 ± 0.07</td>
<td>0.93 ± 0.08</td>
<td>0.77 ± 0.10</td>
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<tr>
<td>10</td>
<td>0.51 ± 0.10</td>
<td>0.74 ± 0.07</td>
<td>0.49 ± 0.06</td>
<td>0.68 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>1:1000</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control sera are all background range.  
<sup>b</sup> AMA titer by IMF against Hep-2 cells.

FIGURE 3. Proposed in vivo pathway for the addition of halogenated xenobiotics. Reductive cleavage of the lipoic acid S, S bond followed by nucleophilic addition to a xenobiotic agent (X) delivers a cyclic adduct (K<sup>X</sup>).
other immunotoxic potential, including a selective stimulation or inhibition of components of the immune system (6). Such effects, of course, would be independent of any modification of autoantigens.

We postulate that people genetically predisposed to PBC have inherited such predisposition based on either the cytochrome p450 pathway or another metabolic process responsible for degrading halogenated compounds. A large number of common pharmaceuticals, such as diuretic agents, are halogenated structures. In fact, halogens are common substituents in pharmaceuticals that modulate binding activity and metabolism. In addition, there are large numbers of detergents commonly used at home and commercially that are rich in halogenated derivatives. Estrogens have already been shown to modulate the expression of many liver-metabolic pathways and may explain the preponderance of women with PBC. Finally, the presence of primarily small bile duct destruction may be reflective of the local mucosal immune response, which is more prominent on epithelial surfaces (36). Indeed, PBC is often referred to as an epithelitis with involvement not only of bile ducts, but also of salivary glands.

Finally, one should discuss the role of xenobiotics in the induction of AMA and their relevance in the disease process. The destructive phase of PBC appears to be mediated locally by an intense mucosal immune response. High titer IgA PDC-E2-specific autoantibodies have been found in the bile, saliva, and even the urine of patients with PBC (37, 38). Moreover, there is unique staining of the cell surface of both bile duct and salivary gland epithelial cells with mAbs to PDC-E2 that colocalizes with IgA transcytosing the cell, suggesting that these may be IgA-PDC-E2 complexes (38, 39). Furthermore, the precursor frequency of PDC-E2-specific CD4 T cells is 100- to 150-fold higher in liver than in the peripheral blood of patients with PBC (17). In addition, we have recently observed the presence of PDC-E2 peptide-specific MHC class I-restricted CD8 T cells only in patients with PBC. Chronic intense PDC-E2-specific responses, either individually or in concert, contribute to the overall pathology. It is ironic that the liver, known to contribute to the induction of tolerance, is precisely the organ that is the target of a central breakdown in tolerance. Toward that end, the influence of these xenobiotics on cellular immune responses in PBC is currently underway. Defining the precise sequence and the molecular basis by which xenobiotics initiate the cascade of autoimmune responses is the next challenge for understanding the etiology and pathogenesis of PBC.

![FIGURE. 4. AMA reactivity to Kx peptide. * Values for Kx peptide represent an average of six patients. For this peptide N-X/N-A = 11.9/12.6 in the same notations as Fig. 1. ](http://www.jimmunol.org/)

### References


