表1　AMAによって認識されるミトコンドリア抗原

抗原	およその分子量（kD）	PBC血清で認識される頻度（%）
PDC-E2	74	95
BCOADC-E2	52	53～55
OGDC-E2	48	39～88
PDC-E1a	41	41～66
E3 binding protein	55	95

余談だが，私は，AMAの対応抗原がPDC-E2その他の2-OADC蛋白であることを1987～1989年にかけて明らかにしていったカリフォルニア大学デービス校のGershwin教授から，AMA対応抗原発見の内情を直接伺ったことがある．ラット肝由来のcDNAライブラリーを患者血清によりスクリーニングし，まずPDC-E2を同定した彼は，某社からpurified PDC-E2蛋白を購入し，イムノブロットを行った．すると，予想どおりPDC-E2の分子量である74 kDのあたりにバンドが認められ，AMAのPDC-E2に対する反応性が裏づけられたが，驚くことに他にもいくつか分子量を異にするバンドがみられた．純粋なPDC-E2であればバンドは1本しかみえないはずなのに，複数のバンドがみられたことから，彼は某社のPDC-E2試薬は純粋なものではなく，ミトコンドリア由来でやはりAMAによって認識されるPDC-E2類似蛋白がこの試薬に混入しているのではないかと推測したという．これがBCOADC-E2，E3BPなどの自己抗原の発見の端緒になった，と，Gershwin教授はやや得意げに話していた．私も，これがscienceか，と感心したのをよく覚えている．

3　AMA陰性PBC，あるいはいわゆる「Autoimmune cholangitis」

AMAがPBC患者に極めて特異性の高い自己抗体であることは周知の事実である．しかし，時に臨床像・血液生化学検査・肝組織像からはPBCが強く疑われるにもかかわらず，AMAが陰性となる症例に遭遇する．このような非典型的な症例をどのように考えたらよいのか，ここ10年ほど論争のテーマになっている．

Autoimmune cholangitisという名称はこの過程で登場してきたもので，AMA陰性のPBCをひとつの疾患概念とし，AMA陽性のPBCと区別して捉えたものである[1]．しかし，その後の研究では，AMA陰性のPBCには血液生化学的にも，病理学的にも，また治療上もAMA陽性のPBCと比較して際立った特徴はみられず，ことさらautoimmune cholangitisという独立した疾患概念を立てる必要はない，とする報告が大勢を占めている[2~4]．ここ数年もAMA陰性PBCに関する報告が散見するが，autoimmune cholangitisという疾患概念の独立性を主張するものはみられない．Stoneらは AMA陰性のPBCにはHLA-DR β 1*08，HLA-DQ β 1*04の頻度がAMA陽性PBCに比し有意に少ないと報告している[5]．しかしこの結果はAMA陰性PBCの独立性を主張するというより，両者のAMA産生能の差がこの結果によって説明できるかもしれないと解釈する方が妥当だろう．近年の英文論文の中で，AMA陰性PBCに対しAutoimmune cholangitisという用語を使用しているのはSherlockの流れを汲むイギリスKings Collegeの研究者だけであり，

彼らはAMA陽性PBCと比べてautoimmune cholangitisでは門脈域の浸潤リンパ球にCD3陽性細胞が有意に多いと報告している[6]．しかし彼らでさえ，その論文のintroductionではautoimmune cholangitisはAMA陽性PBCと臨床的にはほとんど区別できないと述べている．

そもそも，Autoimmune cholangitisという疾患概念を強く主張していたのはイギリスのSherlockであり，彼女の有名な肝臓病学の教科書Diseases of the Liver and Biliary Systemの最新版(第11版，2002年)には「第14章PBC」の中に独立してAutoimmune cholangitisという項が設けられている．しかしここでも，autoimmune cholangitisは生化学的・組織学的にはAMA陽性PBCと区別がつかず，予後もおそらく変わりがない，と述べられている[7]．一方，やはり肝臓病学の教科書として著名なアメリカ・マイアミ大学のSchiffらによるDiseases of the Liverの最新版(第9版)では，PBCのみならず肝臓病学専門家として名高いHeathcoteがPBCの章を書いているが，ここにはAMA陰性PBCについての記載はあるものの，autoimmune cholangitisという語は全く用いられていない[8]．

以上みてきたように，Autoimmune cholangitisという用語は，もはや学会発表や学術論文には使用するべきではなく，AMA陰性PBCと述べる方が妥当であろうと思われる．

4 AMAは本当に「陰性」なのか？

ただ，AMA陰性PBC，というときに，どのような検査によってAMAを検索しているか留意する必要がある．通常の臨床では「抗ミトコンドリア抗体」はラット腎切片を用いた間接蛍光抗体法によって測定され，「抗ミトコンドリアM2抗体」はリコンビナントPDC-E2，BCOADC-E2，OGDC-E2蛋白を抗原としたELISA法によって測定されている．AMAの対応抗原の組換え蛋白を用いたELISAの方がAMAの特異性が高く，かつ感度にも優れているとされている．われわれは以前，血液生化学的・組織学的にPBCと診断された191例の血清を用い，間接蛍光抗体法とPDC-E2・BCOADC-E2・OGDC-E2組換え蛋白を抗原としたELISA法・イムノブロット法のAMA検出感度を比較したことがある[9]．通常の間接蛍光抗体法によるAMAの検出は191例中161例(84％)にとどまっていたが，ELISAでは183例(96％)，イムノブロット法では188例(98％)でAMAが検出され，ELISA法・イムノブロット法の高い検出感度が示された(表2)．ちなみに，現在臨床応用されているELISA法ではこれほどの高い検出感度は期待できない．したがって，研究室レベルの測定法を用いると，AMA陰性と考えられていた症例でも微量のAMAが検出され，AMA陽性となる場合がある，ということは念頭に置くべきである．

表2　間接蛍光抗体法，ELISA法，イムノブロット法によるAMA検出頻度の比較
(文献9より改変)

	AMA陽性	AMA陰性
間接蛍光抗体法	161 (84％)	30 (16％)
ELISA法	181 (96％)	10 (4％)
イムノブロット法	188 (98％)	3 (2％)

ちなみに，今年に入りイタリアのグループからやはりわれわれの仕事と同様，間接蛍光抗体法とELISA，イムノブロットによるAMAの検出感度を比較した論文が発表された[10]．彼らのデータによれば，HEp-2細胞を用いた間接蛍光抗体法のAMA陽性率が

72％，ELISAが81％，イムノブロットが78％であり，ELISA・イムノブロットの方が陽性率が高いもののわれわれのデータよりかなり検出感度は低下している．この原因は明らかではないが，使用した抗原の違いに起因している可能性が考えられる．すなわち，彼らはELISAではヒトPDC-E2，ウシBCOADC-E2，ラットOGDC-E2のB細胞エピトープを並べたハイブリッド組換え蛋白，イムノブロットではウシ心筋由来のミトコンドリア分画を抗原として使用しているが，われわれはいずれでもヒトの組換え蛋白を使用しており，このためわれわれのアッセイ系では高い陽性率が得られたのかもしれない．なお，彼らの論文にわれわれの仕事が引用されておらず，両者の結果の差異についての考察が全くなされていないのはやや遺憾ではある．

5 AMAはどのようにして産生されるのか？

AMAが認識している抗原はミトコンドリア内膜に存在する蛋白である．この細胞内蛋白に対する自己抗体がどのようにして産生されるのか，これもいまだ明らかではない．われわれは以前，AMAが血中のみならず胆汁，唾液，さらに尿中にも存在すること，それも分泌型IgAタイプのAMAが存在していることをイムノブロット法により確認し，AMAが胆管上皮や唾液腺上皮，尿管上皮などの粘膜面から管腔内に分泌されていることを示した[11]．AMAは大腸菌由来のミトコンドリア蛋白をもT細胞・B細胞レベルで認識することが知られており，そのことを踏まえると次のような魅力的な仮説へと導く．

すなわち，粘膜面に存在する大腸菌その他の外来抗原が粘膜上皮から粘膜下組織へと吸収され，そこで樹状細胞により抗原提示がなされ，大腸菌由来ミトコンドリア抗原に特異的なT細胞が活性化される．ここで大腸菌・ヒトの間の分子相同性（molecular mimicry）により，活性化されたT細胞はヒトのミトコンドリア抗原をも認識し，これによってヒトミトコンドリア抗原特異的なB細胞が活性化され，全身の粘膜下組織へと流入しそこで形質細胞へと変化し，分泌型IgAタイプのAMAを産生，これが上皮細胞内を輸送されて粘膜面へと分泌される．この仮説の本質は，粘膜下組織で外来抗原と免疫系とが出会い，molecular mimicを通じてやはり粘膜下組織で自己抗原特異的リンパ球が活性化されるという点，すなわち免疫反応の「場」についての仮説を提示した点にある．しかし，粘膜下組織におけるAMA産生B細胞の実在，ヒトミトコンドリア抗原の粘膜下組織での抗原提示のメカニズムなど，未解明の点は多い．

一方，近年前述のGershwinのグループが精力的に取り組んでいるのがAMA産生における有機合成物質など生体異物（xenobiotics）の関与，より明確に述べれば自己抗原に対するトレランス破綻における生体異物の関与である．そもそもPBCという疾患は，近年疾患に対する認識が高まったということを割り引いてもここ数十年増加の一途をたどっており，しかもイギリス，アメリカ，そして日本など，いわゆる先進国に多く，発展途上国には少ないとされる．おそらくこのあたりからヒントを得て，先進国の環境に豊富に存在する有機合成物質がPBCの発症に一役買っているのではないかという仮説が立てられたものであろう．先に述べたように，従来PDC-E2に対するトレランスの破綻のメカニズムについては大腸菌やレトロウイルスなどの微

生物由来の抗原を介する分子相同性が重視されてきたが，それに代わり，化学物質を介する分子相同性がトレランスの破綻に重要な役割を果たしているのではないかというのが彼らの主張の骨子である．

PDC-E2 の B 細胞エピトープ（抗体認識部位）は inner lipoyl domain と呼ばれる 10 個ほどのアミノ酸であるが，そのほぼ中心にはリジン残基が存在し，このリジンに生体内では通常リポ酸が結合している．AMA はリポ酸の結合したもの，結合していないもの双方を認識することが知られている．というより，もっと正確に述べると，PBC 血清中の AMA は PDC-E2 の inner lipoyl domain 内のさまざまな部位を認識する異なった抗体の混合物であり[12]，リポ酸を認識する AMA もあれば，認識しない AMA もある，ということだろう．Gershwin らはまず，このリポ酸を 3 次構造の類似したさまざまな有機合成物質 18 種で置換し，PDC-E2 ペプチドと有機合成物質とが結合した“ハイブリッド”分子を作成して，AMA との反応性を解析したところ，AMA は 18 種のうち 3 種の合成物質で置換したものを認識し，中にはもともとの PDC-E2 よりもより強く反応するものもあったという[13]．次いで彼らは，この実験で AMA によって認識された 6-bromohexanoate をハプテン，BSA をキャリアとしてウサギを免疫し，PBC 患者血清にみられるものと同等の免疫学的性質を持つ AMA が，免疫したウサギ全例の血中に出現することを確認することに成功した[14]．これらの結果は確かに彼らの主張するように，化学物質に対する免疫反応が PDC-E2 の側鎖であるリポ酸への反応を介して PDC-E2 全体に対するトレランスの破綻につながっていく可能性を示唆するものであると考えられる．

6 AMA は胆管病変に関わっているのか？

AMA は PBC に極めて特異性の高い自己抗体であり，AMA が PBC の特徴的な病理像である胆管破壊（慢性非化膿性破壊性胆管炎）に直接関与しているのではないかと考えるのは当然の発想であろう．しかし，結論からいうと AMA が直接胆管障害に関わっているという証拠はいまだ見い出されてはいない．

AMA そのものが胆管を障害するメカニズムについて論じている報告はさほど多くない．最近，Matsumura は，PBC 患者血清から IgA 型の AMA を抽出し，これを poly-Ig レセプターをトランスフェクトした MDCK 細胞と培養したところ，MDCK 細胞中のカスパーゼ活性が上昇し，その上昇は IgA-PDC-E2 抗体価と強い相関を示した，と報告している[15]．これは IgA 型の AMA が胆管上皮細胞中を輸送される際にアポトーシスによる細胞障害を引き起こすという仮説の傍証となろう．一方，AMA が直接胆管を障害しているのではないと考えられる理由は，主に臨床データから得られる．PBC 患者のうち多くの患者は，UDCA 投与以外治療らしい治療は行わないにもかかわらず無症状のままで大きな進行はみられないという，いわゆる無症候性 PBC である．厚生労働省・難治性の肝疾患調査研究班の報告によれば，無症候性 PBC のうち 70 %の症例はその後 15 年にわたって無症候性のままとどまるとされている．しかし，無症候性 PBC，また症状を伴い進行する症候性 PBC ともに通常は AMA 陽性であり，AMA 抗体価に差はない．症候性 PBC の方が有意に AMA の抗体価が高いのであればともかく，両者に差がないのであれば，AMA の多寡は疾患の進行，そしてお

そらく胆管障害には無関係であると考える方が妥当であろう．また先に詳述したように，AMA 陰性 PBC は AMA 陽性 PBC と臨床像に差はないこと，加えて頻度は少ないものの自己免疫性肝炎(AIH)でも AMA 陽性となることがあるが，このような症例では PBC に特徴的な胆管病変はみられないこと，この2点も AMA の存在が胆管障害に直接結びつくものではないという主張を支持する．加えて，前述の有機化学物質である 6-bromohexanoate を免疫し，PBC 患者と同等の AMA 産生をみたウサギでも，PBC にみられるような胆管障害が生じたとは記載されておらず(おそらくそのような病変は全く出現しなかったと思われる．出現したのであれば論文中に大きく記載され，投稿する雑誌も変わったであろうから)，AMA 単独で胆管病変をもたらすことはどうもなさそうである．

ただ，もちろんこれほど PBC という疾患に特異性の高い自己抗体が胆管障害に全く関係ないということではない．AMA そのものが，ではなく，AMA に代表されるミトコンドリア内膜に存在する 2-OADC 蛋白への免疫反応が胆管障害に関連している，という表現の方が妥当であろう．すなわち，AMA はそれ単独で胆管障害を引き起こす「ツール」ではなくて，胆管障害をもたらす自己免疫反応が起こっているということの「マーカー」である，と考えた方がよいと思われる．

7 おわりに

以上，ここ数年の報告を中心に，PBC における AMA についての諸問題について述べた．PBC という疾患はまだまだわからないことが数多く存在する疾患であるが，この拙稿が本特集号の他の優れた総説とともに，PBC の謎を解くために少しでも貢献できれば幸いである．

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Review

Cellular immune response in primary biliary cirrhosis

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Abstract

The generation of immune responsiveness to self-antigen can result in the pathogenic autoimmune damage of tissues mediated by both humoral and cellular immune responses. Primary biliary cirrhosis (PBC) constitutes a model of autoimmune disease reflective of other organ-specific autoimmune feature. Although the etiology of PBC remained elusive, growing data suggest the role of cell-mediated immune response in the pathogenesis of PBC. Indeed, autoreactive CD4 as well as CD8 T cells have been characterized and their epitopes defined. Molecular mimicry is implicated in the initiation of these autoreactive T cell responses. Moreover, selective enrichment of NKT cells in the liver of PBC is demonstrated using CD1d tetramer.

In this review, we shall focus on the recent advance of cell-mediated immune responses in PBC, which may be directly associated with inflammatory response in PBC.

Keywords: Primary biliary cirrhosis; Mitochondrial autoantigens; Helper T lymphocytes; Cytotoxic T lymphocytes; NKT cells

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune cholestatic liver disease characterized by the presence of anti-mitochondrial antibodies (AMAs) and intense biliary inflammatory response. The major mitochondrial antigens recognized by AMAs have been defined as the E2 components of pyruvate dehydrogenase complexes (PDC-E2) [1]. While autoantibodies to ubiquitous intra-cellular antigens are present in this disease, the reason for the specificity of damage to the intra-hepatic biliary epithelial cells (BECs) remains unknown. Immunohistochemical examination of the cells infiltrating the portal tracts of livers in PBC revealed a predominance of activated lymphocytes [2], which are likely to be involved in the effector functions of cell-mediated immunity and thought to play an important role in the development of inflammation in PBC.

The specificity of bile duct destruction, together with the portal tract lymphoid infiltration and aberrant expression of HLA molecule on BECs [3,4], suggests that intra-hepatic biliary ductular epithelial cells are direct targets of an intense and highly focused immune response. It has been speculated that the destruction of the biliary tract in PBC is mediated by autoreactive CD4 as well as CD8 T cells because of the enrichment of these autoreactive T cells in the liver of PBC [5,6]. Although the events that provoke initial activation remain unknown, molecular mimicry is one of the hypotheses that can explain autoreactive T cells. We have also shown that anti-PDC-E2 antibodies may form immune complexes with antigens and be taken up by antigen presenting cells (APCs), including dendritic cells (DCs) [7]. Recent technology also allowed us to analyze the frequency of natural killer T (NKT) cells in PBC based on their T cell receptor (TCR) engagement [8]. The autoreactive $CD4^+$ and $CD8^+$ T cells, as well as innate immune response such as NKT cells, may collectively form an orchestrated autoimmune effector response that leads to the pathogenesis of PBC (Fig. 1 and Table 1).

Abbreviations: PBC, primary biliary cirrhosis; AMAs, anti-mitochondrial antibodies; PDC-E2, E2 components of pyruvate dehydrogenase complexes; BECs, biliary epithelial cells; APC, antigen presenting cells; DCs, dendritic cells; NKT, natural killer T; TCR, T cell receptor; CTLs, cytotoxic T lymphocytes

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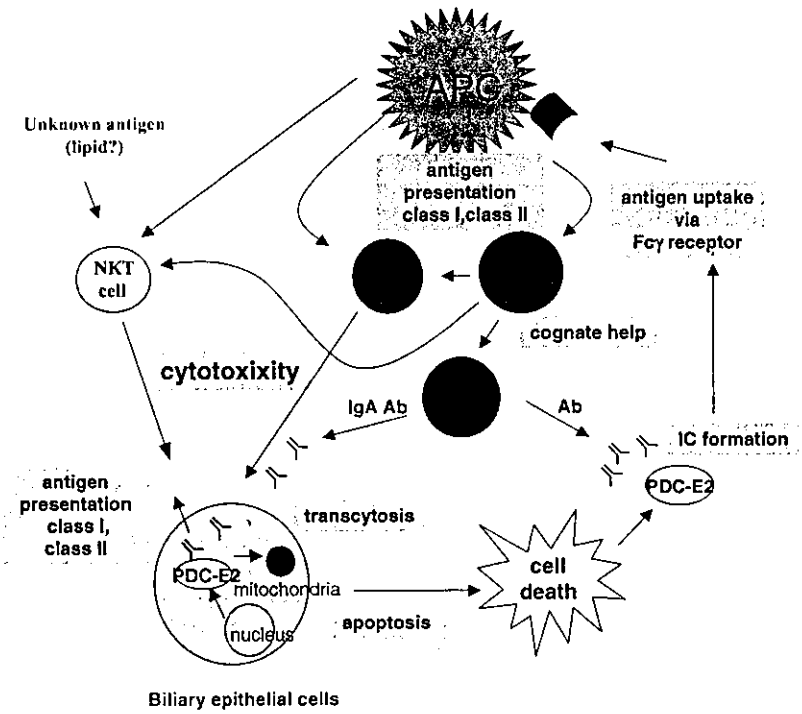

Fig. 1. Scheme of the biliary cell damage induced by the cellular immune response.

2. Role of CD4 T cells in PBC

In disease states which involve tissue destruction and immune mediated cell lysis, the identification and characterization of autoreactive T lymphocyte responses, and the nature of the peptide and the MHC encoded restricting element, is an important step in defining the role of these cells in disease pathogenesis. There have been several reports on the characterization of autoreactive T cell lines in PBC. Van de Water et al. obtained T cell lines from the liver biopsies from PBC patients and demonstrated for the first time that clones reacting with PDC-E2 were present in the livers of patients with PBC [9]. These T cells were $CD4^+$, $\alpha\beta$ TCR^+ and produced IL-2 specifically in response to those antigens. PDC-E2 specific autoreactive CD4 T cell clones were also obtained from peripheral blood by exposing the cells to PDC-E2 [10]. Epitope mapping studies indicated that 54% of patients had cells that proliferated to the outer lipoyl domain while 36% of patients had cells that proliferated to the inner lipoyl domain. Van de Water's studies were done using bovine PDC-E2 and although PDC-E2 is highly conserved across mammalian species, this study did not exclude the possibility that the responses being measured were xenogeneic in nature. Jones et al. [11] used native human PDC-E2 and was able to confirm that T cells responses to this antigen are indeed uniquely associated with PBC.

Shimoda et al. have developed PDC-E2 specific helper T cell lines from peripheral blood lymphocytes [12]. The minimal autoepitopes recognized by these clones were all located within the same region of PDC-E2 peptide 163–176 (GDLLAEIETDKATI) within the inner lipoyl domain of human PDC-E2. The autoepitope almost completely overlaps the B cell epitope, and also include the lipoyl-lysine residue located at amino acids residue 174. The HLA restriction molecules for this epitope were all identified as HLA DRB4*0101. Recently, Shimoda et al. have further evaluated

Table 1
Frequencies of PDC-E2 specific T cells in PBC blood and liver

Cell type	Frequency in peripheral blood	Frequency in liver	Fold increase in liver
MHC Class II restricted T cells specific for peptide 163–176 [5]	$3.66 \pm 0.9 \times 10^{-7}$	$2.53 \pm 0.98 \times 10^{-5}$	69.1
CTLs specific for peptide 159–167 [6]	$3.58 \pm 3.26 \times 10^{-5}$	$4.14 \pm 0.95 \times 10^{-4}$	11.6

The precursor frequencies of MHC Class II restricted T cells and CTLs specific for PDC-E2 in patients with PBC appear to be higher in the liver compared to the blood. MHC Class II restricted T cells and CTLs are 69.1 and 11. 6 times more prevalent, respectively, when compared to their frequencies in blood [5,6]. All data are expressed as the mean ± standard deviation.

the frequency of the precursor cells of the HLA DRB4*0101 restricted autoreactive T cell recognizing PDC-E2 peptide 163–176, isolated from peripheral blood mononuclear cells (PBMC), regional hepatic lymph nodes, and from the liver of patients with PBC [5]. Results showed a disease-specific 100–150-fold increase in the precursor frequency of PDC-E2 163–176-specific T cells in the hilar lymph nodes and liver when compared with PBMC from PBC patients.

3. Role of CD8 T cells in PBC

Autoreactive $CD8^+$ T cells are thought to be involved in the pathogenesis of several autoimmune diseases [13–15]. Previous investigations have demonstrated the accumulation of antigen-reactive $CD8^+$ T cells at the site of the inflammation in several human autoimmune diseases as well as in murine models of human autoimmune diseases [15–18]. Identification of the autoantigen peptide, that is the target of MHC Class I restricted cytotoxic T lymphocytes (CTLs) in PBC is an important initial step not only in determining the mechanism by which tolerance to the self peptide is abrogated, but also in facilitating the potential for therapeutic strategies.

Kita et al. have characterized the first MHC Class I restricted epitope for PDC-E2, namely amino acid 159–167, a region very similar to the epitope recognized by MHC Class II restricted $CD4^+$ cells and by antibody [7]. This peptide, amino acids 159–167 of PDC-E2, induces specific MHC Class I restricted $CD8^+$ CTL lines from 10/12 HLA-$A2^+$ PBC patients, but not from controls, following in vitro stimulation of peripheral blood lymphocytes with antigen pulsed dendritic cells (DCs). These PDC-$E2_{159–167}$ specific CTLs have shown to specifically lyse target cells pulsed with PDC-$E2_{159–167}$ peptide as well as targets infected with recombinant vaccinia virus expressing PEC-E2.

Furthermore Kita et al. recently found a 10-fold increase in the frequency of PDC-$E2_{159–167}$ specific CTLs in the liver as compared to the blood in PBC by using tetramer technology [6]. The precursor frequency of the CTLs in blood was significantly higher in early stage PBC. These data, for the first time, document the enrichment of autoantigen-specific $CD8^+$ T cells in the PBC liver, suggesting that $CD8^+$ T cells play a significant role in the immunopathogenesis of PBC.

By using limiting dilution analysis (LDA), the frequency of peripheral $CD4^+$ helper T cells specific for the PDC-E2 163–176 epitope in PBC patients was estimated to be 3.66×10^{-7}, while that in the liver was 1.66×10^{-5} to 4.13×10^{-5}, a 100-fold enrichment in the liver as compared to PBMC's. Taken together, these studies indicate that there is selective enrichment for both PDC-E2 specific $CD4^+$ and $CD8^+$ autoreactive T cells localized to the site of tissue injury in patients with PBC. The discrepancy of the level of frequency between autoreactive $CD4^+$ and $CD8^+$ T cells could be due to the difference of the methods of detection. Interestingly, the extent of enrichment in the PDC-E2 specific autoreactive $CD4^+$ T cells is higher than $CD8^+$ T cells. This difference cannot be readily explained by the differences in the techniques utilized in the previous and present study. On the other hand, if indeed the values are in fact true differences, this could reflect an increasing role for $CD4^+$ T cells in the pathogenesis of PBC. Further study on $CD4^+$ T cells using Class II tetramer is warranted and may provide additional information about the nature of the autoreactive $CD4^+$ T cells.

The combination of tetramer staining with flow cytometry-based functional assays for T cells, e.g. intra-cellular cytokine staining assay, allows the direct evaluation of the effector functions of antigen-specific $CD8^+$ T cells [19–21]. Specifically, such assays have been used to identify viral and tumor-specific $CD8^+$ T cells that are non-functional [20,22]. We demonstrated that autoreractive CTLs in PBC were functionally heterogenous in terms of IFN-g production [6]. Although the implication of this finding is not clear at this time, there is no doubt that elucidating the effector functions of autoreactive T cells is critical for a complete understanding of the nature of PBC.

In addition to binding the cognate peptide presented by MHC molecule, antigen-specific T cells have also been shown to be capable of recognizing analogue peptides with slightly different amino acid sequences. These peptides have the potential to significantly alter the functional consequences of the mediated activation, as exemplified in both mice and humans [23–28]. Peptides carrying amino acid substitutions at the T cell receptor (TCR) contact residues of the peptides may induce T cell non-responsiveness or anergy through TCR antagonism as a consequence of partial or incomplete activation [29]. In order to seek for analogue peptide antagonizing effector function of CTLs specific for this autoantigen, the effector functions of the PDC-E2 specific CTLs against alanine-substituted peptides were evaluated [30]. An alanine substitution at position 5 of this epitope significantly reduced peptide-specific effector functions of CTLs. Moreover, this analogue peptide inhibited effector functions of the CTLs to the prototype peptide, including cytotoxicity and IFN-g production. These data suggest that a modification of the immunodominant autoepitope can be utilized to manipulate the CD8 T cell responses against the autoantigen PDC-E2.

4. Dendritic cells and cross-presentation

Dendritic cells (DCs) are professional antigen presenting cells, which is capable of stimulating naive T cells and initiate primary immune responses. DCs are also likely to be important for the induction of immunological tolerance and for the regulation of immune responses. Immunohistochemical study showed increased number of inter-digitating cells as well as highly restricted distribution of $CD83^+$ DCs in PBC, suggesting the role of DCs in PBC [31]. PDC-E2

specific CD4 T cells are induced more efficiently by using antigen pulsed DCs [32].

The term "cross-presentation" has been used to describe the uptake and re-presentation of exogenous antigens primarily in the MHC Class I pathway. Professional APCs, such as DCs, macrophages, and B cells are capable of intra-cellular processing of exogenous antigen to generate peptides presented by MHC Class I [33,34], the exogenous antigen being presented by the same APCs to both $CD4^+$ and $CD8^+$ T cells [35]. DCs in murine models have been shown to mediate internalization of antigen-immunoglobulin complexes (immune complexes, ICs), and promote efficient MHC Class I as well as Class II-restricted antigen presentation [36]. In terms of MHC Class II presentation, Fcγ receptors, which bind ICs represent a privileged antigen internalization route for efficient MHC Class II-restricted antigen presentation in DCs [37].

PDC-E2 specific autoreactive CTLs appear primed in vivo only in PBC patients. Thus, we hypothesized that cross-presentation is involved in the maintenance and/or amplification of CTL response against PDC-E2, with PDC-E2-specific autoantibody playing a key role; i.e. PDC-E2 specific autoantibodies may capture PDC-E2 antigens released from dying cells to form PDC-E2 immune complexes (ICs), which may facilitate the uptake of PDC-E2 antigen through Fcγ receptors (FcγRs) on professional antigen presenting cells (APCs).

Indeed, PDC-E2 specific CTLs are generated by pulsing DCs with full-length recombinant PDC-E2 protein. Furthermore, using soluble PDC-E2 complexed with either PDC-E2 specific human monoclonal antibodies or affinity purified autoantibodies against PDC-E2, the generation of PDC-E2 specific CTLs, occurred at 100- and 10-fold less concentration respectively, compared to soluble antigen alone [7].

These evidences suggest that autoantigens can be captured by the autoantibodies and subsequently presented via Class I pathway with high efficiency. The findings for the first time defines a unique role for autoantibodies in the pathogenesis of an autoimmune disease.

5. Role of NKT cells in PBC

Natural killer T (NKT) cells are a subset of lymphocytes incriminated in playing an important role in the modulation of the innate immune response and the development of autoimmunity. Considerable evidences suggest that NKT cells are implicated in human as well as murine model of autoimmune diseases [38]. However, there have been only limited studies attempting to quantitate the number of NKT cells in autoimmune disease including PBC, particularly because of difficulties associated with definition of this subpopulation. Kita et al. used a human CD1d (hCD1d) tetramer produced by a baculovirus expressing recombinant CD1d protein complexed with α-galactosylceramide (α-GalCer) and quantitated hCD1d tetramer reactive cells in blood and liver from controls and patients with PBC [8]. The frequency of CD1d-αGalCer-restricted NKT cells was similar between blood and liver in healthy individuals. By contrast, the frequency of CD1d-αGalCer-restricted NKT cells in the liver was significantly higher than in the blood of PBC patients. The frequency of CD1d-α-GalCer-restricted NKT cells in the liver was also significantly higher in PBC patients than healthy individuals. Selective enrichment of CD1d-αGalCer-restricted NKT cells at the site of inflammation is observed in PBC, suggesting a role of these cells in the development of PBC. The precise role of NKT cells in the development of PBC, however, remains an enigma. A possible explanation is that the unique inflammatory environment in PBC liver causes enhanced apoptosis of CD1d-αGalCer-restricted NKT cells recruited to the liver, which may contribute to the inflammation, while the continuing trafficking of the NKT cells from the circulation reduces the number of such cells in the periphery. Alternatively, a reduced frequency of CD1d-αGalCer-restricted NKT cells in the blood of PBC may be associated with lack of a protective role in the progression of PBC. Further studies focusing on the CD1d-αGalCer-restricted NKT cells in the liver of early stage PBC will help define these issues.

6. Molecular mimicry in PBC

Molecular mimicry between host autoantigens and unrelated exogenous proteins is one of the hypothesis used to explain how immune response to self-proteins arise, break tolerance, and lead to autoimmune disease. Microorganisms produce a multitude of foreign antigens that collectively comprise the major set of determinants recognized by the immune system. Specifically, activation of autoreactive T cells by these mimicry epitopes may be crucial to the pathogenesis of PBC.

Evidences of molecular mimicry at a T cell level stem from reports showing mimicry epitopes from *Borrelia burgdorferi* in Lyme-arthritis and from *Chlamidia pneumoniae* in autoimmune inflammatory heart disease [39,40]. PDC-E2, particularly its inner lipoyl domain, is highly conserved between bacteria, yeast and mammals [41]. It is possible to propose that the autoimmune phenomena in PBC result from T cell epitopes of microbial proteins being mimicked by peptides.

Shimoda et al. has demonstrated the presence of molecular mimicry at the T cell clonal level between human and *Escherichia coli* PDC E2 [12]. The common essential amino acids of the epitope for HLA DRB4*0101 restricted autoreactive T cell recognizing PDC-E2 peptide 163–176 were E, D, and K at positions 170, 172, and 173, respectively (EXDK motif). Cross-reactivity of T cell clone with exogenous antigens from *E. coli* PDC-E2 peptide with EXDK sequence has been shown. However, cross-reactivity of T cell clone has been shown to neither the self-peptides derived from human

glycogen phosphorylase b nor the HLA DRα chain, both of which contain some amino acid homology with human PDC-E2 163–176 but not contain EXDK sequences.

This is a demonstration of "molecular mimicry" at the T cell clonal level. It can be hypothesized that PBC may have a bacterial etiology. In this hypothesis, the T cells first recognize the lipoyl domain of *E. coli* PDC-E2. Then, these T cells cross-react with self peptide from HLA DRα chain or human PDC-E2 by aberrantly expressed Class II HLA molecules on biliary epithelial cells. Finally, this initiates the autoimmune cascade leading to the destruction of the bile ducts in which AMAs and/or autoreactive T cell specific for mitochondrial antigens may play a pathogenic role. At this stage, the autoimmune process takes place in the absence of the exogenous antigens, such as *E. coli* PDC-E2, which initiated the immune response. Shimoda et al. further evaluated the role of molecular mimicry by analysing 30 kinds of mimicry peptide with EXDK-sequences, and found seven mimicry peptides derived from microbial proteins [42]. Various pattern and degree of activation by mimicry peptides was shown in each T cell clone, suggesting a diverse spectrum of autoreactive T cells reacting to a single epitope of human PDC-E2.

Molecular mimicry was also analyzed with CD8 T cell epitope. To investigate the potential role of molecular mimicry in CD8 T cell in the pathogenesis of PBC, Kita et al. carried out studies aimed at identifying naturally occurring peptides for the 159–167 peptide of PDC-E2 that may serve as agonists [30]. Alignment algorithms was used to search for amino acid homologies between PDC-E2 159–167, the newly identified MHC Class I restricted epitope, and microbial proteins and agonistic effect of these homologous peptides on the PDC-E2 specific CTLs was also assessed. PDC-$E2_{159–167}$-specific CTLs cross-reacts with a partially homologous peptide derived from *Pseudomonas aeruginosa*.

7. Future prospective

PBC is believed to be a multifactorial disease [43]. It is characterized by autoreactive B cell response, or AMA, as well as CD4 and CD8 T cell responses targeted at the autoantigen PDC-E2. In addition to the previously defined epitope, autoreactive T cell response in PBC may also involve other epitopes on PDC-E2 restricted by the same HLA molecule, as well as epitopes restricted by other HLA molecules. Thus, a comprehensive mapping of the T cell epitope repertoire on the PDC-E2 molecule may provide further clues regarding the role of PDC-E2 specific T cells in the pathogenesis of PBC.

The mechanisms involved in the breakdown of self-tolerance is one of the most important issues in defining the basis of PBC. Despite great progress in our understanding of the pathophysiology of PBC, etiology is the question that remained unresolved. Although there are numerous theories proposed, mostly incorporating the interaction of genetic predisposition and environmental agents, there are sparse data that help in establishing a specific causation. The hypothesis of molecular mimicry is one of the most attractive theory which implies that foreign pathogens with homology to self-protein or modified self-protein can break tolerance. Several reports have suggested the association of autoimmune diseases with drugs, chemicals, and other environmental factors. Specifically, many xenobiotics are metabolized in the liver. Liver autoantigens exposed to these chemicals could be modified and become immunogenic. It would be intriguing to study about the effect of the environmental xenobiotics as an initiating factors that leads to the loss of tolerance to self-proteins in genetically susceptible hosts, resulting in development of PBC.

Therapeutic applications of these serious recent progresses in the field of cellular immune response in PBC are very important and should be taken into consideration. The discovery that certain peptides can exert TCR antagonism on the epitope specific T cells has provoked considerable interest in their potential use as therapeutic use of the antagonistic peptide. In addition, future progress for the characterization of DCs as well as NKT cells in PBC may also provide a therapeutic potential by manipulating these cells.

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Autoimmunity and environmental factors in the pathogenesis of primary biliary cirrhosis

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It is generally believed that autoimmune processes are initiated when tolerance to self-proteins is broken. Primary biliary cirrhosis (PBC) is an autoimmune liver disease of unknown etiology. Autoimmune attack in PBC is predominantly organ-specific, despite the presence of mitochondrial autoantigens, the major targets of autoimmunity in PBC, in all nucleated cells. Although the events that provoke initial activation remain unknown, the hypothesis of molecular mimicry implies that foreign pathogens with homology to self-protein or modified self-protein can break tolerance. Several reports have suggested the association of autoimmune diseases with drugs, chemicals, and other environmental factors. Specifically, many xenobiotics are metabolized in the liver. Liver autoantigens exposed to these chemicals could be modified and become immunogenic. We propose that exposure to the environmental xenobiotics is one of the initiating factors that leads to the loss of tolerance to self-proteins in genetically susceptible hosts, resulting in development of PBC.

Keywords: autoimmunity; environmental factors; pbc; xenobiotics

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Introduction

Although autoimmune diseases are characterized by both humoral and cellular immunity against self-antigens, the exact pathophysiology of these diseases remains an enigma. It is generally believed that both heritable traits and environmental factors can affect the susceptibility to autoimmune diseases. Primary biliary cirrhosis (PBC) is an autoimmune cholestatic liver disease characterized by the presence of anti-mitochondrial antibodies (AMAs) and intense biliary inflammatory response. Despite progress in understanding of the underlying mechanisms for PBC, its etiologic origins remain largely unknown. Genetically inherited factors are thought to be important for the development of PBC. The risk factor for developing PBC in a first-degree relative is 100-fold more common, and the onset of disease in the relatives is often within a few years of the other's diagnosis (1). Jones et al. have also reported that the sibling relative risk of PBC in the city of Newcastle-upon-Tyne was 10.5, and similar to values seen in other autoimmune diseases (2). On the other hand, epidemiological studies also suggest the role of environmental factors in triggering or exacerbating PBC (3, 4). Studies examining the effect of migration also suggest the role of environmental factors (5). There are several geographical studies of PBC which are controversial (6, 7). Infection is one of the most important environmental factors associated with autoimmunity. Several data suggest the role of infective agents in the pathogenesis of PBC, although no pathogenic agent has been defined to be clearly implicated in the etiology of PBC (8).

A major advance towards defining the pathogenesis of PBC initiated from identification of the mitochondrial proteins targeted by anti-mitochondrial antibodies (AMAs). The primary mitochondrial antigen recognized by AMAs has been defined as the E2 component of the pyruvate dehydrogenase complex (PDC-E2) (9). In addition, autoreactive CD4 as well as CD8 T cells recognizing PDC-E2 have been identified in PBC and their epitopes defined as well. Nevertheless, it is not clear what events initiate the recognition of the self-proteins as immunogenic despite several attractive hypotheses which have been proposed (10, 11).

Molecular mimicry has been used to describe a spectrum of antigenic cross-reactivities thought to

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Abbreviations and acronyms

2-OADC	2-oxo acid dehydrogenase complexes
AMAs	Anti-mitochondrial antibodies
BCOADC	Branched chain 2-oxo dehydrogenase complex
CTLs	Cytotoxic T lymphocytes
OGDC	2-oxoglutarate dehydrogenase complex
PBC	Primary biliary cirrhosis
PDC	Pyruvate dehydrogenase complex
TFA	Trifluoroacetyl

Key messages

- Environmental factors are thought to trigger autoimmunity in genetically predisposed individuals, leading to the development of PBC, which is characterized by autoreactive B cell and T cell responses.
- Pathogens with homology to the PDC-E2, the major autoantigen of PBC, may induce autoimmunity by molecular mimicry.
- Exposure to environmental chemicals, or xenobiotics, may generate modified autoantigen, which is recognized as foreign and initiates immune responses against the autoantigen.

underlie autoimmune diseases. Similarities between foreign antigens and host elements could lead to antigenic cross-reactivity by both B and T cells, resulting in the autoimmune responses. The association between autoimmune diseases and drugs, chemicals, and other environmental factors has long been suggested. Growing evidence suggests that modification of autoantigens by chemical compounds can break tolerance and lead to the autoimmune responses. The modified self-proteins may become sufficiently distinct, leading to their recognition by the immune system as foreign. Of special interest is the hypothesis that such immune responses may not only recognize modified proteins but also cross-react with native self proteins, resulting in abnormal cellular and humoral immune responses.

In this review, we will discuss how immunological tolerance is disrupted in PBC, with emphasis on the possible role of xenobiotics as inducing agents for PBC.

Immunological tolerance

Tolerance is the essential normal state of absence of harmful immune responses to body (self) components, and autoimmune diseases may develop when this tolerance is disrupted. Potentially self-reactive T and B lymphocytes are generated during lymphopoiesis in the central lymphoid tissues, thymus and bone marrow, by processes referred to as positive selection, to establish a complete immune repertoire. Afterward a process referred to as negative selection is required to ensure deletion of lymphocytes that recognize self-molecules, which is the basis of central tolerance. The fact that self-antigen is lethal to immature lymphocytes in the central lymphoid organs, yet stimulatory for mature cells in the periphery, is not fully understood. The major components of peripheral tolerance, albeit not fully defined, are ignorance, anergy/deletion and regulation. *Ignorance* refers to prevention of contact between lymphocyte and autoantigen, usually accomplished by sequestration of autoantigens by cell membranes or vasculature, but also by cell death through apoptosis. Ignorance is also applicable to potentially autoreactive T cells existing in a non-activated or naive state, which is mediated by cell surface adhesion molecules that direct their recirculation to lymphatic channels rather than into tissues, thus precluding their contact with cognate autoantigens (12). *Anergy* refers to a counterproductive state of unstable metabolic arrest when lymphocytes are rendered non-reactive rather than activated after antigenic contact (13). This typically occurs when lymphocytes receive their first signal (antigen contact) but are deprived of a necessary additional signal, called a second signal (14) or co-stimulatory signal (15). The co-stimulatory signal is usually delivered by receptor-ligand interaction. *Regulation* describes the capacity of one class of lymphocyte to curtail the induction and/or effector functions of another class, in either an antigen-specific or non-antigen-specific manner. Regulation may depend on a cytokine-mediated deactivation of a T cell response, and/or deviation from a type 1 (Th1-T cell mediated) destructive-inflammatory pattern of cytokine expression towards a type 2 (Th2-T cell mediated) humoral-regulatory pattern. Regulatory T cells still remain uncharacterized, but studies on autoimmune mouse models implicate thymus-derived cells with one or another of two particular markers, either markers of NKT cells or the CD25 marker. A recent paper also suggests that a subset of the dendritic cells can contribute to the expansion and differentiation of T cells that regulate or suppress other T cells (16, 17).

Autoreactive immune responses in PBC

As discussed in the previous section, both central and peripheral tolerance comprise a mechanism of deleting dangerous immune responses to the self-components. Central tolerance is efficient, notwithstanding autoreactive cells do still 'leak through' to the

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periphery, either because their cognate autoantigen was lacking or their affinity with it in central lymphoid tissues was too low to promote deletion. Indeed, considerable evidence suggests abnormal immune responses in PBC, involving both antibody responses and cellular immune responses, in which self-components are perceived as foreign by the immune system (18). Advances in molecular biology have played an important role in identifying the autoantigens and the epitopes targeted by AMA in PBC. The mitochondrial antigens recognized by AMA have been identified as E2 components of the 2-oxo acid dehydrogenase complexes (2-OADC): pyruvate dehydrogenase complex (PDC), 2-oxoglutarate dehydrogenase complex (OGDC), and branched chain 2-oxo dehydrogenase complex (BCOADC) (19). The most predominant reactivity of AMA is directed against PDC-E2, which contains two lipoic acid-binding regions, the inner and outer lipoic acid-binding regions, sharing a sequence homology.

Autoreactive T cells have also been characterized in PBC. PDC-E2 specific autoreactive CD4 T cell clones were obtained from liver as well as from the peripheral blood by *in vitro* stimulation of intrahepatic or peripheral lymphocytes to PDC-E2 (20, 21). Using PDC-E2 specific CD4 T cell lines derived from peripheral lymphocytes, the minimal epitope on PDC-E2 was defined (22). The autoepitope almost completely overlaps the B cell epitope and includes the lipoyl-lysine residue located at amino acids residue 174. Shimoda et al. have further evaluated the frequency of the human lymphocyte antigen (HLA) DRB4*0101 restricted CD4 autoreactive T cells recognizing PDC-E2 peptide 163-176 (23). Results showed a PBC-specific 100–150-fold increase in the frequency of PDC-$E2_{163\text{-}176}$-specific CD4 T cells in the hilar lymph nodes and liver when compared with that in periphery.

Cytotoxic T lymphocytes (CTLs) are thought to be directly involved in the tissue injury in PBC. We have characterized the first major histocompatibility complex (MHC) class I restricted epitope for PDC-E2, namely amino acid 159-167, a region very close to the epitope recognized by MHC class II restricted CD4 cells and by antibody (24). In addition, using tetramer technology we recently found a 10-fold increase in the frequency of PDC-$E2_{159\text{-}167}$ specific CTLs in the liver as compared to the blood in PBC patients (25).

Several hypotheses have been proposed to explain the induction of these autoreactive responses in PBC (Table 1). When the host is suffering from an infection, normal immune responses may lead to the destruction of infected cells, thereby releasing cryptic proteins that would otherwise never be seen by the immune system. The term 'cryptic' was initially referred to the proteins that are normally hidden from immune recognition. The newly encountered proteins are perceived as foreign by the immune system, thereby initiating autoimmunity. Now, cryptic antigen is also known to be applicable at the level of particular autoepitopes within certain autoantigens (26). Because presentation of T cell antigen usually requires antigen processing, the mechanism of antigen degradation has a profound influence on the type of epitope presented to T cells. Increasing evidence suggests that the revelation of cryptic self-epitopes may be a crucial factor for the initiation of autoimmunity (26). Indeed, certain chemicals are reported to modify autoproteins and disturb antigen processing so that cryptic self-epitopes are presented (27). Another hypothesis, namely 'molecular mimicry' is discussed in the next section.

Molecular mimicry in PBC

Molecular mimicry between host autoantigens and unrelated exogenous proteins is one of the hypotheses used to explain how autoantibodies to self-proteins as well as autoreactive T cells arise, break tolerance, and lead to autoimmune disease. Although the etiology of autoimmunity is still unknown, numerous studies have implicated infectious agents as triggering events in the breakdown of self-tolerance. Microorganisms produce a multitude of foreign antigens that collectively comprise the major set of determinants recognized by the immune system. These antigens potentially include a wide variety of carbohydrates, lipids, and proteins that can be recognized by specific receptors of inflammatory cells. Evidence of molecular mimicry stems from reports showing mimicry epitopes from *Borrelia burgdorferi* in Lyme-arthritis and from *Chlamydia pneumoniae* in autoimmune inflammatory heart disease (28, 29). Moreover, evidence has been reported for the induction of autoimmune disease by viral infection through molecular mimicry in herpes stromal keratitis (30). Molecular mimicry is well summarized (31–34) and also exemplified in the several autoimmune diseases including Guillain-Barre syndrome (35), type 1 diabetes (36), and myocarditis (37) in the recent issue. Thus, it has been proposed that the autoimmune phenomena in PBC are also resulted from both B cell and T cell epitopes of microbial proteins being mimicked by peptides.

Although there are no solid data supporting the

Table 1. Hypothetical etiology of PBC

Genetic factors
Environmental factors
Infection – Exposure of cryptic epitopes from killed cells
Infection – Molecular mimicry
Modification of autoantigen by environmental chemicals

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role for microbial agents in the etiology of PBC (8, 38), molecular mimicry for an extrinsic protein of an infectious agent has long been suggested as a possible initiating event in PBC. PDC-E2, particularly its inner lipoyl domain with a lipoated lysine residue, is highly conserved between bacteria, yeast and mammals (39). AMA has been shown to react with both human and bacterial mitochondria (40). Some evidence suggests the cross-reactivity of autoantibodies with infectious agents in PBC. The reactivity of PBC sera with an extract of Mycobacterium gordonae, and the reactivity of antibodies to the M gordonae 65 kDa heat-shock protein with the mitochondrial antigen in PBC has been demonstrated (41, 42). Recent data using PCR analysis of PBC liver have failed to show a unique bacterium, including Helicobacter species (43, 44). Jones et al. have also suggested the role of bacterial motif DNA as an adjuvant for the breakdown of tolerance to the PDC molecule in a murine experimental model (45).

Shimoda et al. have demonstrated the presence of molecular mimicry at the T cell clonal level between human and *E. coli* PDC E2 (22). PDC-$E2_{163-176}$-specific CD4 T cells derived from different PBC patients recognize common essential amino acids E, D, and K at positions 170, 172 and 173, or EXDK sequence, of the autoantigen. Cross-reactivity of these T cell clones with *Escherichia coli* PDC-E2 peptide, which also has an EXDK sequence, has been documented.

Molecular mimicry in PBC has also been analyzed at the CD8 T cell level. Kita et al. used alignment algorithms to search for amino acid homologues between PDC-$E2_{159-167}$, the newly identified MHC class I restricted epitope, and microbial proteins. Agnostic effects of these homologous peptides on the PDC-E2 specific CTLs were assessed. PDC-$E2_{159-167}$-specific CTLs cross-react with a partially homologous peptide derived from *Pseudomonas aeruginosa*. Taken together, these results provide preliminary evidence for the role of molecular mimicry in the development of PBC, at the levels of B cell, CD4 T cell and CD8 T cell autoimmunity. The mechanisms involved in the breakdown of self-tolerance are one of the most important issues in defining the basis of PBC. Breakdown of tolerance to PDC molecules at a B-cell level has been shown in a murine experimental model. However, the breakdown of T-cell tolerance to PDC was hard to achieve (46). Nevertheless, autoreactive T cells are identified from PBC blood and their epitope defined. Further study regarding how T-cell tolerance to PDC is broken in PBC is certainly warranted.

Chemical exposure and autoimmune diseases

In addition to the infectious agents as possible candidates of molecular mimicry, exposure to drugs and environmental chemicals has long been speculated as another initiation factor for autoimmune diseases (Table 2). Various connective tissue diseases have been linked with chemical exposures. The association between silica exposure and scleroderma has been reported. Occupations with continuous exposure to silica dust, such as miners, are known to have a higher risk of developing systemic sclerosis (47, 48). Several drugs, such as bleomycin and cocaine, are known to induce scleroderma-like diseases (49, 50). Systemic lupus erythematosus (SLE) is another disease that could be induced by certain drugs in genetically predisposed individuals. Indeed, various drugs can induce a SLE-like syndrome, or 'drug-induced lupus', characterized by slowly developed and relatively restricted autoimmune responses, mainly directed against histones and denatured DNA. Although the mechanisms by which drugs induce or exacerbate lupus remains unclear, several hypotheses, including interference DNA methylation by drugs, have been proposed (51). Other environmental factors with potential association with SLE include silica dust, hair dye, and water contaminated with metal cleaning solvents (52). High doses of estrogen are also known to provoke flares of SLE (53). Certain estrogen-like substances present in pesticides or in foods like yams may contribute to the development of SLE. In addition, several reports have suggested the association between cosmetic breast implants, especially with silicone, and connective tissue diseases, although this is still a controversial issue. Finally, two kinds of diseases, toxic oil syndrome and eosinophilia-myalgia

Table 2. Association of autoimmune diseases with environmental chemicals

Diseases	Environmental exposure
Scleroderma	Silica
Scleroderma-like diseases	Bleomycin, cocaine
Systemic lupus erythematosus (SLE)	Drugs, estrogen, silica dust, hair dye, metal cleaning solvents, pesticides (estrogen like substances), yams (estrogen like substances)
Connective tissue diseases	Silicone breast implants
Toxic oil syndrome Eosinophilia-myalgia syndrome	Consumer products
Autoimmune thrombocytopenia	Cocaine
Autoimmune thyroiditis	Polychlorinated biphenyls, polybromated biphenyls

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syndrome, are known to be induced by the ingestion of consumer products. Clinical feature of these diseases were similar to connective tissue diseases and immunosuppressive therapy was used for the treatment of these diseases. Although those with clear association of chemical exposure account for quite a small proportion of all cases with autoimmune disease, these cases can provide a clue to elucidate the pathogenesis of such diseases. In animal models, autoimmune diabetes can be induced with multiple low doses of the pancreatic toxin streptozotocin (54). This disease has shown to be T cell-mediated (55). Multiple injection of $HgCl_2$ can also induce autoimmune diseases in animals (56).

The underlying mechanisms for the induction of autoimmune responses and diseases by these chemical exposures remain unknown. These chemicals may cause autoimmune responses indirectly by their toxic effects on the tissues, resulting in the release of auto-antigens. Some of them may facilitate abnormal MHC expressions on various cells. Alternatively, they may directly act on the lymphocytes, favoring the production of certain cytokines. Both mercury and gold in rodents can induce immunological disorders by activating signal transduction pathways that result in the expression of cytokines. Furthermore, of special interest, growing evidence suggests that modification of the autoantigen by chemical compounds can break tolerance and lead to the autoimmune responses.

Modification of auto-antigens by environmental chemicals

An essential function of the immune system is to distinguish between self and non-self. This discrimination is indispensable to prevent the host from destructive immune attack as a consequence of recognition by the immune system. Lymphocytes include the major properties of memory, specificity, and discrimination between self and non-self. Both B cells and T cells display numerous repertoires in terms of antigen recognition. Although the exact mechanism of this discrimination has not been fully characterized, this mechanism is likely to be relative rather than absolute and compromise may occur in specific circumstances such as chemical modification of the autoantigens, which may culminate in the autoimmune manifestation.

Table 3. Different environmental agents/processes that could be suspected in PBC

Infections
Chemicals and xenobiotics
Medicinal drugs
Contraceptive pills
Foods
Vaccines
Deficient sunlight/vitamin D
Pregnancies/microchimerism

Xenobiotics are foreign compounds that may either alter or complex to defined self-proteins. They present in the environment in forms of industrial chemicals, drugs and even foods, which we may be exposed to. Xenobiotics may exhibit direct toxicity to the vital cellular targets, and thus are usually eliminated either in the urine or in the bile. In addition, some xenobiotics may induce changes in the molecular structure of the native proteins sufficient to induce an immune response. Such immune responses may be promiscuous in their ability to recognize either the modified or unmodified native protein (57, 58). For example, some metals can induce autoimmune reactions by creating new high affinity sites for MHC determinants on self-epitopes (56). The chronic presence of the self-protein serves to perpetuate the immune response initiated by the xenobiotic-induced adduct and leads to autoimmunity (59, 60). For example, in murine models, SLE can be elicited with an isoaspartyl-modified form of self-antigen (61).

While the direct toxic activity of the xenobiotics has been well analyzed, their adverse effects on the immune system, especially those affecting the liver, have been appreciated only in recent years. The liver is an important organ for metabolism/degradation for xenobiotics and an altered immune response (62). 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 auto-antigens within the liver could lead to a breakdown of tolerance and induction of a self-reactive response that is liver-specific. There is evidence based on *in vivo* studies in guinea pigs exposed to halothane that Kupffer cells carry trifluoroacetylated protein adducts (63). These protein adducts are not found in other organs, including hilar lymph nodes, suggesting that the generation of autoreactivity to this protein adduct is likely caused by a local liver response. In fact, a liver-specific autoimmune disease can be observed in some patients exposed to chlorofluorohydrocarbon anesthetics (64, 65). Halothane is an anesthetic that may lead to severe hepatitis on very rare occasions. Halothane is transformed, by oxidation with cytochrome P450 into CF_3COC_1, a very reactive intermediate that may bind to cellular proteins and particularly to several microsomal proteins, to form trifluoroacetyl (TFA) proteins (66). These reactive metabolites can be identified by using anti-TFA antibodies. CYP2E1 is also a target of this reactive metabolite both in humans and in rats (67, 68). It is important to note that immunization with halothane induces the formation of antibodies that cross-react with not only the haptenated TFA immunogen, but

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also to lipoated PDC-E2, the major autoantigen of PBC (69, 70). This finding has important implications in the pathogenic mechanisms associated with PBC, an autoimmune disease marked by the presence of AMA (10, 71).

PDC-E2, a major autoantigen in PBC, is essential for the function of mitochondria by using lipoic acid to rapidly transition between an oxidized and reduced state. Porcine PDC-E2 loses antigenicity when lipoic acid is removed (72). The higher antigenicity of lipoated PDC-E2 could be explained by its higher accessibility to modification by exogenous agents (e.g., xenobiotics), thus providing neoantigens that can be recognized by the immune system (73).

We hypothesized that the lipoic acid residue of PDC-E2 serves as a xenobiotic target which, following the modification of the lipoyl lysine residue, becomes immunogenic (Fig 1). Actually, it is plausible that liver enzymes, including cytochrome P450, may transform specific chemicals into reactive molecules that may bind to the lipoyl lysine residue, making immunogenic neoantigens. We further hypothesized that the AMA response is induced by the modified self-protein and that the antibody specificities present in such sera include those that recognize the xenobiotic modification. To test this hypothesis, we use a microbead system for which peptide synthesis, derivatization, and determination of antibody reactivity can all be performed on the same solid support (74). We took advantage of *ab initio* quantum chemistry and synthesized the inner lipoyl domain of PDC-E2, replacing the lipoic acid moiety with synthetic structures designed to mimic a xenobiotically-modified lipoyl hapten, and quantitated the reactivity of these structures with sera from PBC patients (75).

Interestingly, AMA from all seropositive patients with PBC, but not from 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 (75). For the first time these results show that an organic compound can serve as a mimeotope for an autoantigen. The fact that chemical modification of a native protein results in higher antigenicity of the protein provides evidence for a potential mechanism by which environmental organic compounds may be a causative agent of PBC. Interestingly, immunization of rabbits with such a xenobiotic organic compound,

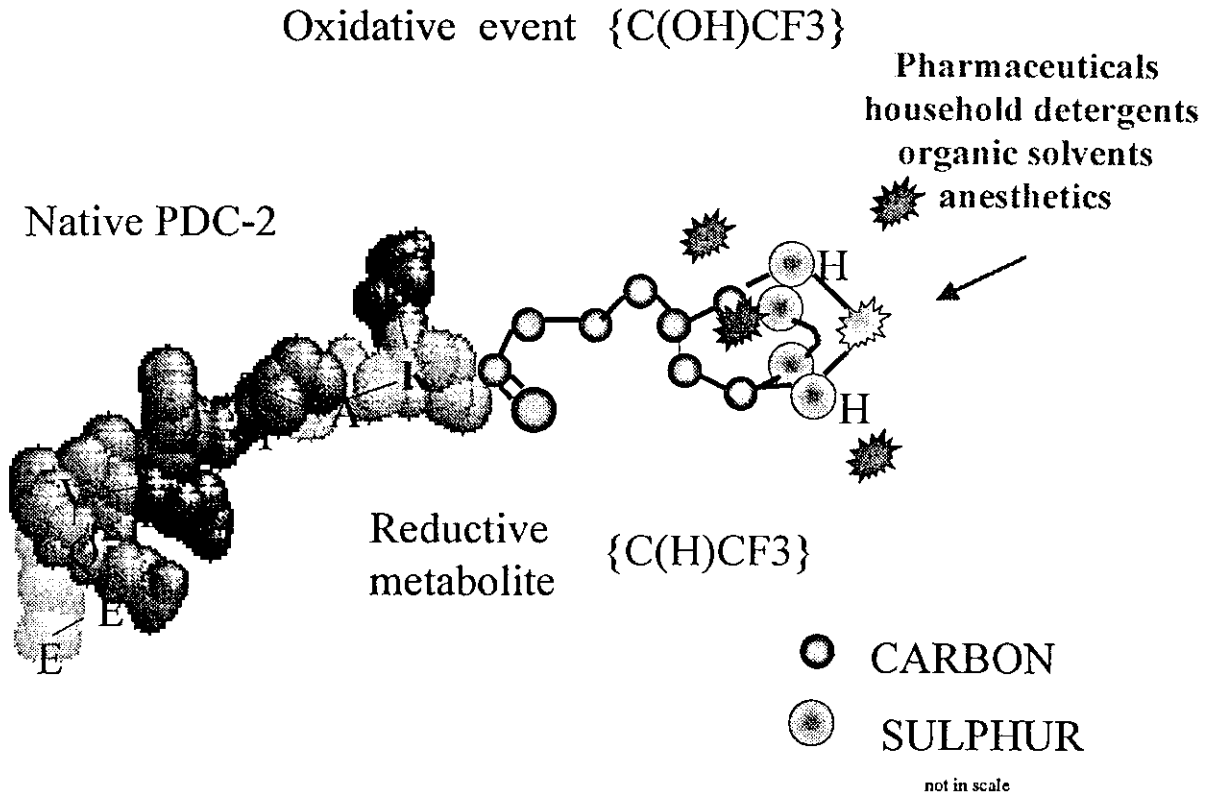

Figure 1. Modification of the lipoic acid residue of PDC-E2.

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6-bromohexanoate, coupled to bovine serum albumin (BSA) resulted in the production of anti-mitochondrial antibodies (76). This evidence further reinforced the notion that environmental xenobiotic agents can be a risk factor for the induction of PBC.

Conclusion

PBC is believed to be a multifactorial disease. It is characterized by autoreactive B cell response, or AMA, as well as CD4 and CD8 T cell responses targeted at the autoantigen PDC-E2. Identification of the cause that breaks self-tolerance and initiates these autoreactive immune responses is the key to understanding the etiology of this enigmatic disease. Environmental factors, including microorganisms and xenobiotics are thought to initiate the development of autoimmunity in genetically predisposed individuals. Various bacterials have been shown to carry epitopes with homology to human proteins to a different extent. They may serve as targets of immune response during an infection, which may cross-react with the host self-protein, breaking the self-tolerance barrier. On the other hand, environmental chemicals may modify host autoantigen to form neoantigen with a stronger immunogenicity, resulting in the breakage of tolerance. The two factors may have a synergetic effect on the host immune system. For example, a xenobiotic may modify a bacterial homologue to form a neoantigen. Exposure to the neoantigen during an infection may result in a greatly enhanced immunogenicity in the inflammatory environment. Careful dissection of these different mechanisms is the challenge that must be addressed to understand the pathogenesis of PBC.

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