

Review

The role of *E. coli* infection in the pathogenesis of primary biliary cirrhosis

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Abstract. Among various infectious agents possibly involved in the pathogenesis of primary biliary cirrhosis (PBC), *Escherichia Coli* (*E. coli*) has received special attention because of epidemiological and experimental evidence linking this bacterium with the disease's development. This review discusses early and more recent epidemiological studies associating recurrent urinary tract infections with *E. coli* and the development of PBC. We also critically review data provided over the years demonstrating disease-specific humoral and cellular immune responses against *E. coli* antigens in patients with PBC. Finally, we assess the relevance of experimental findings reporting cross-reactive immunity between mimicking sequences of *E. coli* and the major PBC mitochondrial antigens in the pathogenesis of the PBC. We also address the extent to which molecular mimicry and immunological cross-reactivity can be considered as a critical pathogenic process linking infection with self destruction.

Keywords: Autoimmunity, autoimmune disease, bile ducts, cholestasis, liver, immunity, tolerance, mimicry, cross-reactivity

List of abbreviations

PBC	primary biliary cirrhosis
AMA	anti-mitochondrial antibody
BEC	biliary epithelial cell
<i>E. coli</i>	<i>Escherichia coli</i>
OADC	oxo-acid dehydrogenase complex
PDC	pyruvate dehydrogenase complex

1. Introduction

Much work has been devoted to understanding the mechanisms involved in the orchestrated and tissue-specific autoimmune attack against the biliary epithelial cells (BEC) of the small intrahepatic bile ducts, which

is a characteristic feature of primary biliary cirrhosis (PBC) [1–8]. PBC is a cholestatic liver disease which mainly affects middle-aged women [1,9]. The disease progresses over time and the bile duct assault leads to the development of cirrhosis and subsequent liver failure [1,9,10].

The mechanism(s) responsible for PBC development remain unknown [3,11]. As in other autoimmune diseases, various infectious agents have been implicated as possible triggers and perpetrators of self-destructing immunopathogenic processes [4,7,12–28]. The most studied of these in the case of PBC is that of recurrent urinary tract infections (UTI) with *E. coli* [7,8,12,17,20,21,29–32]. This review aims to discuss the evidence involving *E. coli* in the pathogenesis of PBC. As there are limited data to suggest that *E. coli* is an hepatotropic bacterium, most of the discussion will be focused on the epidemiological and immunological findings linking the anti-microbial response with PBC's pathogenesis.

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2. Immunological features of primary biliary cirrhosis

PBC is a puzzling autoimmune disorder [3,11]. The disease is characterised by a prominent female preponderance (> 95%), presentation after the fourth decade of life (>80% of the cases), lack of the disease in childhood, frequent co-existence of extrahepatic autoimmune manifestations such as autoimmune thyroid and rheumatological diseases (up to 70% of cases), and a generalized immune dysregulation expressing itself in the form of elevated levels of IgM and seropositivity for disease-specific autoantibodies in the great majority of the cases (> 95%) [1,10]. There is no solid evidence to suggest that patients with PBC respond to immunosuppression and the current treatment includes administration of ursodeoxycholic acid, an endogenous bile acid which is effective in improving biochemical cholestatic markers [1,9,10].

2.1. Diagnostic and prognostic significance of AMA

The serological hallmark of PBC is the presence of antibodies against mitochondrial antigens (AMA) mainly of IgG, but also of IgA and IgM isotype [33]. The association of AMA with PBC is so striking that the disease is questioned in their absence [1,34–37]. In fact, most of the cases reported as seronegative for AMA do indeed have mitochondrial-specific autoreactivity when more sensitive techniques are used for their detection [35–43]. Longitudinal studies have shown that virtually all asymptomatic women who are incidentally found positive for AMA in the absence of biochemical markers of cholestasis and clinical evidence of the disease, do in fact have histopathological features of PBC, and will develop full-blown disease over time [44,45].

2.2. The role of cellular and humoral autoimmunity in PBC

PBC is characterised histologically by nonsuppurative destructive cholangitis in the presence of a lymphocyte-rich mononuclear cell infiltrate, which has been immunohistochemically shown to be comprised of CD8, CD4 and B cells in the periductular areas, especially in early-stage PBC [1,46]. Evidence suggestive of an orchestrated autoimmune response against BECs includes an aberrant expression of disease-specific mitochondrial antigens on the apical surface of the biliary epithelium at the prodromal, pre-clinical phases

of the disease, followed by upregulation of major histocompatibility complex (MHC) class II antigens and adhesion molecules [11]. Disease-specific T cell (CD4 and CD8) and B cell responses against mitochondrial components have been documented, and their investigation has led to the identification of the major autoantigenic targets and characterization of their dominant autoepitopes [4,47–50].

2.3. The oxo-acid dehydrogenase complexes as targets of AMA

Early studies investigating the antigen specificity of AMA have shown that these antibodies are directed against members of the 2-oxo-acid dehydrogenase complex (OADC) family of enzymes (formerly known as M2 antigens) [33,51]. AMA mainly recognizes the E2 subunits of pyruvate dehydrogenase complex (PDC), branched-chain 2-oxo acid dehydrogenase complex (BCOADC) and 2-oxoglutarate dehydrogenase complex (OGDC) [33,51]. More than 95% of patients with PBC react with PDC-E2, 20–70% of them recognize BCOADC-E2 or OGDC-E2, and to a lesser extent other subunits of OADC antigens such as PDC-E1 α and PDC-E1 β [33,41,42,51].

The E2 subunits of the PDC-E2, BCOADC and OGDC are evolutionarily highly conserved, both between species and between the different complexes [52–54]. Each of these three enzymes occupies a key position in the energy metabolism within the cell. PDC links glycolysis to the Krebs cycle, OGDC is in the Krebs cycle itself and BCOADC is involved in the regulation of the oxidation of the branched-chain amino acids. Each complex consists of multiple copies of at least three enzymes (E1, E2 and E3); the E2 subunit is the structural core of these complexes, to which multiple copies of E1 and E3 subunits are non-covalently bound [1]. The E2 enzymes have a common structure, which consists of the N-terminal domain containing the lysine-bound lipoyl groups, which have a central role in the catalytic cycle; the peripheral subunit binding domain is responsible, at least in part, for binding the E1 and E3 components together; and the C-terminal inner core, which houses the active enzymatic site responsible for the acyltransferase activity. E3 is common to all 3 complexes whereas E1 and E2 are unique to each complex. PDC contains a fourth polypeptide with a structural role, the E3 binding protein (E3BP), once termed protein X [1,33,48,51].

All PBC sera reactive with PDC-E2 also react with the PDC-E3BP antigen [48,51,55]. Inhibition studies

based on recombinant PDC-E2 and PDC-E3BP antigens have revealed the two antigens to be targets of B-cell cross-reactive responses, with one being able to inhibit reactivity against the other [55,56]. More recent studies have suggested that the initial breakdown of self tolerance is to PDC-E2, with epitopes spreading to E3BP [5]. Less than 50% of patients with PBC have AMA that recognize all three oxo-acid complexes [51].

AMA reactive with PDC-E2, BCOADC-E2 or OGDC-E2 are each directed against a conformational epitope that includes the inner lipoyl domain, but several studies have shown that at a 100-fold higher concentration, AMA can also react with the outer domains of the OADC antigens [1,11,48]. In view of the sequence similarity between inner and outer regions, this differential reactivity has been interpreted as arising from cross-reactivity between the two lipoyl binding domains, the priming immunogenic region being the inner lipoyl domain [1,11,48]. The highly conserved structure in the E2 subunit of 2-OADC and their lipoyl domains indicates that lipoic acid may be part of an immunodominant epitope [57,58]. Studies investigating the contribution of lipoic acid to autoantibody reactivity have, nonetheless, shown that AMA are capable of binding to both lipoylated and unlipoylated PDC-E2 [3,57,58].

Anti-OADC AMA are so disease-specific that they are considered to be relevant to disease pathogenesis [3]. Most researchers have concluded that CD4 and CD8 T cells rather than antibodies *per se* are involved in causing BEC destruction [3,4,37,48]. A profusion of studies in experimental models of PBC have revealed a significant role of CD4 and CD8 T-cells in the induction of pathological features resembling those in man, while early reports investigating the role for AMA have led to the conclusion that AMA have little to do with diseases pathogenesis [2,3,43,59,60]. Thus, it was shown that immunization with recombinant human PDC-E2 can induce high AMA titres in experimental animals, with no evidence of liver damage despite the presence of these autoantibodies for a lengthy period of time [59]. In the clinical setting, there was no universal PBC recurrence following liver transplantation, despite the persistence of high titre of AMA [61]. Early studies have shown that anti-PDC-E2 AMA were able to inhibit the enzymatic activity of PDC-E2 *in vitro*, but there was no evidence to suggest that these antibodies interfere with the enzyme's activity *in vivo* or that they can induce biliary cell damage [11, 62]. More recent studies, however, have provided data indicating that antigen-specific helper T cells, cytotoxic

T cells and B cells act in concert in inflicting damage to BECs in patients with PBC [3].

The first finding in support of this view was that of a striking overlap of the immunodominant B- and T- (both CD4 and CD8) cell epitope on PDC-E2 [16,18, 47,49,50,63]. This region is located within the inner lipoyl-binding domain of the subunit, spanning amino acid 212-231 (PDC-E2₂₁₂₋₂₃₁). Later it was found that soluble PDC-E2 in complex with a PDC-E2-specific human monoclonal antibody, or purified anti-PDC-2 antibodies targeting this epitope, promote the generation of PDC-E2-specific cytotoxic cells at a 100-fold lower concentration than otherwise required in the presence of the soluble antigen alone [50]. These data clearly demonstrate that immune complexes (and in particular those containing autoantibodies against PDC-E2) can be efficiently internalized, processed, and presented by antigen-presenting cells (APCs) to induce specific cytotoxic T lymphocytes in PBC patients [50]. Thus, AMA may play a pathogenic role in facilitating the engagement of a PDC-E2 specific CD8 T cell immune response [33]. Another mechanism by which AMA may participate in an orchestrated attack of BEC has been suggested by a recent study investigating the properties of the PDC-E2 in apoptotic BEC lines [64]. This report showed that PDC-E2 remains immunologically intact in BECs after apoptosis, and localizes within apoptotic bodies where it is accessible to AMAs, and can induce specific cytotoxic T lymphocytes in PBC patients [64,65]. That AMA may have pathogenic properties has been indirectly suggested by a clinical report where two infants who received AMA transplacentally from their mothers, developed liver pathology that lasted as long as the autoantibody persisted in their serum [66].

Determination of the three-dimensional structure of the inner lipoyl domain of human PDC-E2 has revealed that PDC-E2₂₁₂₋₂₃₁ is physically exposed on the molecule surface which may partially explain its particular antigenicity [17,48]. However, the mechanism by which this short sequence becomes the focus of PBC-specific anti-mitochondrial immune responses remains obscure.

Assuming that a concerted autoimmune attack is the ultimate mechanism of damage in PBC, the question as to what triggers this autoimmune attack remains without an answer. A number of putative infectious organisms have been implicated in the pathogenesis of PBC, including *Escherichia coli*, mycobacteria, *Lactobacillus delbrueckii*, chlamydia, *Novosphingobium aromaticivorans* and a betaretrovirus [4,7,12-28]. Most of the

available data are concentrated on the role of *E. coli* in view of epidemiological and immunological findings supporting the notion that this infectious agent is strongly associated with the disease [7,8,12,17,20,21, 29–32,67,68].

3. Epidemiological studies linking *E. coli* to PBC

An early study conducted from investigators at the Royal Free Hospital in London reported an increased incidence of recurrent urinary tract infection in women with PBC as compared with women with rheumatoid arthritis or women with other forms of chronic liver disease [29]. The prevalence of bacteriuria in 19% of women with PBC contrasted with that of 5–6% found in age matched populations of normal women, female patients seen in general practice, or those attending medical outpatients [29]. Over the two year study period, 57% of the bacteriuric PBC patients had more than one bacteriuric episode, and 26% had three episodes or more in less than one year [29]. *E. coli* was cultured in 70% of the specimens [29]. A subsequent study from the Newcastle group has failed to confirm this association [69].

Epidemiological support to the report from the Royal Free Hospital was again provided in 2001 by Parikh-Patel et al. in the first controlled epidemiological analysis showing a positive association of PBC with recurrent UTI [68]. This study carried out in association with patient support groups suggested urinary tract infection as a risk factor for PBC [68]. Parikh-Patel studied 241 PBC patients from the United States, with controls consisting of 261 of their siblings as well as 141 friends without PBC [68]. A significant finding of this report was that female PBC cases were almost twice as likely to have reported UTI as their female siblings [68]. The findings of Parikh-Patel were questioned since cases were recruited from members of an internet-based patient forum (known as “PBCers”), and controls were recruited by patients themselves, leading to the potential for significant control biases.

In 2005, the group of Gershwin reported the findings of the largest recent epidemiological study in patients with PBC [67]. This study included 1032 PBC patients followed up in 20 tertiary referral centres in the USA representative of all but two States, and 1041 demographically-matched controls [67]. It was found that 59% of patients with PBC reported a history of UTI, and UTIs were independently associated with PBC by multivariate analysis.

In keeping with all previous studies, the most recent case-control study published by the Newcastle group has also reported an association between PBC and UTI [70]. In their study, Prince et al. included two cohorts of patients with PBC. The primary cohort consisted of 318 PBC patients from northeast England identified from a survey of consultant hepatogastroenterologists in the region [70]. The second cohort of 2,258 patients with PBC was recruited from members of the UK based PBC Foundation, a national support group of patients with PBC. The control group consisted of 3,936 demographically matched individuals selected randomly from electoral control datasets [70]. Multivariate analysis has shown that UTI were associated with PBC in both groups [70].

4. Experimental studies linking *E. coli* to PBC

An early study by Hopf et al. reported an association between PBC and the presence of rough form mutants of *E. coli* in the patients’ faecal samples [71]. These rough forms have defective polysaccharide synthesis which leads to abnormal, fragile cell walls. The authors studied 21 PBC patients and found that up to 50% of all faecal *E. coli* were rough [71]. A subsequent study by Butler et al. reported a high percentage of bacterial rough forms (approximately 40%) in the urine of patients with PBC [29]. The percentage of rough mutants was similar in PBC and controls suggesting that these rough forms are a generalised feature of recurrent bacteriuria and not specific for PBC [72]. The same group has reported reactivity to PDC-E2, the major PBC autoantigen, in 52% of sera from patients with evidence of chronic UTI but without liver disease [31].

Direct support for a role of *E. coli* in the induction of PBC has been provided recently in an experimental study demonstrating the ability of recurrent UTI to induce PBC-related cholangiopathy in mice [73]. In experimental models of UTI it has been shown that during the infection, uropathogenic *E. coli* (UPEC) invade the urothelial cells to form bacterial communities, which act as a reservoir for recurrent episodes of bacteriuria. These findings have been the basis of a hypothesis suggesting that the autoimmune attack in PBC is triggered by exposure to uropathogenic *E. coli* during recurrent UTI, and is facilitated by internalized bacteria within the urothelial cells of the bladder [73]. To test this, Palermo has followed female C57BL/6 mice receiving transurethral inoculations with uropathogenic *E. coli*, or sterile phosphate buffered saline as controls, for a

Sequence		Identity (%)	Homology (%)
KVGDKVEAEQSLITVEGDKASMEVPS PQAGI VKEI KVS VGDK			
KVG: K: . L : E: DKA: : . : G. : : I V. G :		13/42 (31)	27/42 (64)
KVGEKLS EGDLLAEIETDKATI GF E VQEEGYLAKI L VPE GTR			

Fig. 1. Sequence alignment of the lipoyl domains of *E. coli* and human PDC-E2. The row in between the sequences indicates identities and conservative (:) or semi-conservative substitution (.). Amino acid in standard single-letter code. PDC-E2, pyruvate dehydrogenase complex E2 subunit.

period of 4 months [73]. The findings of this study are of interest. Serum samples obtained from the mice receiving inoculations with uropathogenic *E. coli* developed significant anti-mitochondrial antibody reactivity. Histopathological assessment of these mice also indicated features of non-suppurative destructive cholangitis, including small bile duct destruction with lymphocytic infiltrates and granuloma formation, which are characteristics found in liver biopsies of patients with PBC [73]. These observations suggest that recurrent UTI with uropathogenic *E. coli* can generate an aberrant immune response that specifically targets intrahepatic bile ducts, and is similar to what can be seen at early stages of PBC.

5. Molecular mimicry between *E. coli* and human autoantigens in PBC

We and others have shown that molecular mimicry and immunological cross-reactivity involving infectious agents and autoantigens are characteristic features of gastrointestinal and liver-specific autoimmunity [5,7,8,12,15,21,74–87]. Molecular mimicry between *E. coli* and human PDC-E2 as a mechanism responsible for the breakdown of tolerance to mitochondrial autoantigens, was first suggested by Baum and Burroughs [30]. These authors hypothesised that as E2 polypeptides are evolutionary highly conserved, the similarity between the major PDC-E2 autoepitope and its *E. coli* homologue (Fig. 1) is an essential element of a cascade of immune-reactions, involving an initial immune response against *E. coli* PDC-E2 in women with recurrent UTI, followed by cross-reactive recognition of the human PDC-E2 epitope [30]. This step would ultimately lead to disease-specific pathology and full-blown PBC, with cross-reactivity operating at a B- and/or T-cell level [4,12].

Experimental support for this hypothesis has been provided in subsequent studies. It has been shown that sera from patients with PBC react with both human and *E. coli* PDC-E2, and the epitope of *E. coli* PDC-E2

also maps to similar lipoyl domains [53]. However, the reactivity against human PDC-E2 was approximately 100-fold higher than that to *E. coli* PDC-E2 [88]. Moreover, antibody responses specific for the core epitopic region of human PDC-E2 (PDC-E2_{212–226}) did not cross-recognise the corresponding sequence on *E. coli* PDC-E2, despite the significant degree of amino acid homology between *E. coli* and human PDC-E2 [17,18,27].

As sequence similarity does not necessarily lead to structural/conformational similarity, and therefore need not equate with antigenic mimicry [74], we analysed the degree of structural mimicry of the corresponding inner lipoyl domains of *E. coli* and human PDC-E2 epitopes (Fig. 2A). We noted that at the structural level, *E. coli* and human PDC-E2 are dissimilar at a degree that may explain the inability of anti-human PDC-E2 antibody responses to cross-recognise their *E. coli* homologue and *vice versa*. The analysis of the predicted structure of the CD4 T-cell epitopes on PDC-E2 has shown that these two epitopes are highly homologous at the 3D-level (Fig. 2B). This finding could imply that *E. coli* and human PDC-E2 are targets of cross-reactive responses at the CD4-T cell level. Indeed, Shimoda et al. found that human PDC-E2 autoepitope (GDLLAEIETDKATI) and *E. coli* PDC-E2 (EQSLITVEGDKASM) cross-react at the CD4 T cell level, and that the ExDK motif shared by human and *E. coli* PDC-E2 is essential for T cell epitope recognition [21,49,63]. Further characterisation of cloned T-cells with specificity for the human PDC-E2 autoepitope, has shown that a motif-sharing *E. coli* OGDC-E2 peptide can activate these T-cells [21]. The reverse experiment has been performed based on the assumption that if molecular mimicry is responsible for the initiation of autoimmune responses in PBC, T-cell clones specific for *E. coli* antigens should proliferate in response to self-mimicking mitochondrial autoepitopes [89]. Sixteen independently derived T-cell clones specific for the OGDC-E2 peptide were obtained, and tested for proliferation against the human OADC-E2 autoepitopes from PDC-E2, OGDC-E2 and BCOADC-E2 [89]. Thirteen of the 16 clones

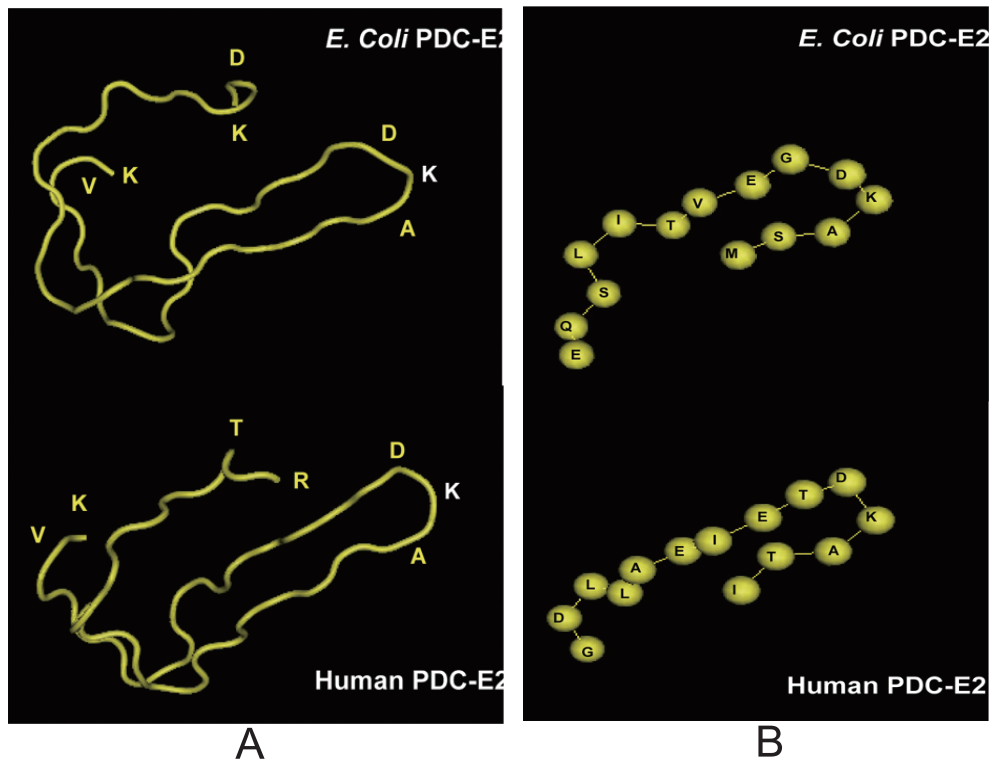


Fig. 2. Three dimensional modelling of the lipoyl domains of *E. coli* and human pyruvate dehydrogenase complex E2 subunit (A) and of the corresponding CD4 T-cell epitopes (B). The first and last two amino acids of the N- and C-terminus of the sequence are presented in yellow in a single letter code. The lipoylated lysine (K) residues are coloured white. The neighbouring residues are also presented (yellow). The structures were analysed with the Cn3D visualization tool with a wire frame displayed in yellow.

responded to the human OADC-E2 peptides but none of these to the control protein [89].

The pathogenic relevance of *E. coli*/human PDC-E2 mimicry and *E. coli* OGDC-E2/human PDC-E2 has been challenged since, as the argument goes, cross-reactive immunity can be readily predicted in view of the highly conserved nature of PDC-E2 among species, and in particular of its inner lipoyl domain. Since the number of micro-organisms possessing OADC homologues of the human enzymatic complex is vast and infection with such microbes frequent, as is anti-microbial OADC reactivity, immune responses against OADC is expected to be equally common. But this is not the case, probably reflecting the opposition of controlling mechanisms to the emergence of self-reactivity.

If the microbial mimics were unrelated to OADC their potential pathogenic role would be more probable. Such OADC unrelated mimicking sequences of the major PDC-E2 autoepitopes have been the focus of recent studies from our group [7,15–18,26,27]. We have shown the existence of human PDC-E2 mimicking sequences on *E. coli* antigens totally unrelated to PDC-

E2 or other OADC antigens (Fig. 3) [17]. The biological significance of these homologies was highlighted by the fact that these *E. coli* sequences are better mimics to the major human mitochondrial PDC-E2_{212–226} autoepitope than the *E. coli* PDC itself [17]. Antibody reactivity with at least one of the OADC unrelated *E. coli* mimics has been documented in two-thirds of the PBC patients, 1/3 of them reacting with the *E. coli* ATP-dependent helicase *hrpA*, and the periplasmic maltose-binding sequences. This reactivity was strongly associated with a history of recurrent UTI [17]. Collectively, these data reveal the existence of *E. coli* mimics of the human PDC-E2 autoepitope as targets of cross-reactive responses at the B- and T-cell level [17].

6. Peculiar antibody reactivity to caseinolytic protease P of *E. coli*

Two studies in Spanish and British patients with PBC have demonstrated the presence of antibodies directed to a short 18-meric sequence within the proteolytic sub-

Species	Protein		Identities	Similarity (%)
Human	Pyruvate dehydrogenase complex-E2	K L S E G D L L A E I E T D K		
<i>E.coli</i>	ATP-dependent clp X	K A S E G E L L A Q V E P E D	8	14/15 (93)
<i>E.coli</i>	ATP-dependent helicase hrpA	<i>L M T D G I L L A E I Q Q D R</i>	7	12/15 (80)
<i>E.coli</i>	Periplasmic maltose-binding protein	<i>G Y A Q S G L L A E I T P D K</i>	7	11/15 (73)
<i>E.coli</i>	Fatty acid oxidation complex alpha	<i>D A A V E D L L A E V S Q P K</i>	6	9/15 (60)
<i>E.coli</i>	(P)ppGpp synthetase II	<i>L A T L D D L L A E I G L G N</i>	6	9/15 (60)
<i>E.coli</i>	Nitrate reductase 2	<i>G Q A M V D L L A E Y E K V G</i>	6	8/15 (53)
<i>E.coli</i>	Pyruvate dehydrogenase complex-E2	K V A A E Q S L I T V E G D K	5	11/15 (73)

Fig. 3. Sequence alignment between the core epitopic region of human E2 subunit of pyruvate dehydrogenase complex (PDC-E2) and its *E. coli* mimics. Amino acids in standard single-letter code. Identities in bold; conservative substitutions in *italic*.

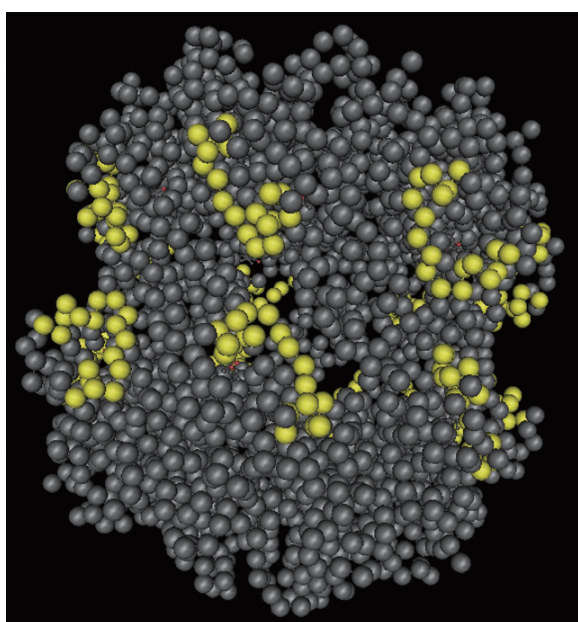


Fig. 4. Three dimensional structure of the hepta-symmetric ring *E. coli* caseinolytic protease P (ClpP) with a space fill backbone (grey). It appears that the immunodominant *E. coli* ClpP₁₇₇₋₁₉₄ B-cell epitope is located in a solvent-accessible surface region of the protein, compatible with antibody binding (displayed in yellow). The structure was analysed with the Cn3D visualisation tool.

unit of the ATP-dependent Clp protease (ClpP₁₇₇₋₁₉₄) of *E. coli* ClpP₁₇₇₋₁₉₄ [19,20]. These antibodies were present in serum of some 33% of patients with PBC but in fewer than 4% in patients with other autoimmune diseases [17,20]. Interestingly, a three-dimensional model of *E. coli* predicts this peptide to be repetitively exposed on the surface of the molecule making it accessible to antibody binding (Fig. 4).

The significance of these findings became apparent when it was recognized that a peptide of *E. coli* ClpX, the regulatory ATP-binding subunit of the Clp complex, has a sequence that is very similar to that of the dominant T-cell epitope of PDC-E2 (14/15, 93%)

(Fig. 3) [17]. Despite its high degree of homology, *E. coli* ClpX is not recognised by serum samples of patients with PBC [17]. This led to the hypothesis that B-cells, internalizing intact ClpP/ClpX complexes by virtue of their receptors recognizing the ClpP subunit, might present as antigen presenting cells a peptide of the ClpX subunit, in association with Class II MHC [90]. If that sequence were the “mimic” of the major mitochondrial PDC-E2 epitope, then PBC-specific help would be given by the CD4⁺ T cells recognizing that epitope, for the production of antibodies corresponding to the B-cell receptor, that is binding ClpP [90]. This would be analogous to what is seen in the immunological response to viruses, where B- and T- cell epitopes may be from totally different parts of the virus particle.

Preliminary work using conventional proliferation and intracellular cytokine staining assays revealed strong T-cell responses of peripheral blood mononuclear cells to *E. coli* ClpX-but not to ClpP epitope in PBC women with recurrent UTI. These proliferative responses were absent in sex and age matched healthy women. Another set of experiments based on intracellular cytokine measurements by flow cytometry, revealed that the frequency of interferon- γ producing CD4 T-cells is similar or higher after stimulation with *E. coli* ClpX₂₈₀₋₂₉₄ than with the major human PDC-E2 autoepitope indicating that the microbial peptide activates the T-cells efficiently as the immunodominant autoepitope. More work is needed to delineate the significance of disease-specific immune responses to microbial Clp complexes in patients with PBC.

Collectively, these findings strengthen the contention that a microbial exposure, especially through recurrent *E. coli*-urinary tract infection, may be instrumental to the appearance and/or the maintenance of anti-mitochondrial responses. It also raises the question of whether autoimmune responses targeting sp100 (the major target of antibodies giving a multiple nucle-

ar dot pattern) and gp210 (the target autoantigen of anti-nuclear pore complex antibodies) might arise by a similar mechanism involving molecular mimicry at the B- or T-cell level. Interestingly, antibody reactivity against the PBC-specific sp100 nuclear autoantigen strongly correlates with AMA seropositivity in rUTI women, with or without evidence of PBC. A study from our group has shown that among the women with recurrent UTI but without liver disease, 8 (80%) of 10 AMA-positive women reacted with sp100 compared with none of the 10 AMA-negative women [32]. Among the PBC patients, 14 (74%) of 19 with recurrent UTI and 1 (4.8%) of the 21 without recurrent UTI reacted with sp100. None of the women with bacteriuria but without liver disease reacted with other disease-specific nuclear autoantigens like gp210 or lamin B receptor [32]. These data add support to the notion that *E. coli* infection may be instrumental for the breakdown of tolerance to mitochondrial and nuclear autoantigens in patients with PBC.

While attractive as models, such mechanisms are still not proven valid beyond any doubt, and most of these processes do not necessarily reflect what may go on in an *in vivo* pathophysiological context at the early stages of the disease. The recent development of credible animal models of PBC will give us the opportunity to investigate the role of *E. coli* infection as a pathogenic trigger of PBC.

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