T-cell autoimmunity in primary biliary cirrhosis

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1. Primary biliary cirrhosis is a chronic cholestatic liver disease with an autoimmune aetiology. The classical histopathological lesion, of portal tract biliary epithelial cell damage, is accompanied by a T-cell-rich mononuclear cell infiltrate and upregulation of cell surface markers suggestive of local T-cell activation and cytokine release. This suggests that T-cell mediated mechanisms play an important role in tissue damage in primary biliary cirrhosis.

2. CD4+ T-cells specific for the E2 component of human pyruvate dehydrogenase complex (PDC-E2), a highly conserved enzyme which plays a critical role in intermediate metabolism, are present in the peripheral repertoire of the majority of patients with primary biliary cirrhosis. These cells are almost entirely absent from normal and chronic liver disease control subjects. The observations that peripheral blood PDC-E2-specific cells are most commonly seen in early stage disease, when active bile duct damage is occurring, and that PDC-E2-specific cells can be found in the portal tract infiltrate at times when this damage is occurring, suggest that these autoreactive cells may have a role to play in the aetiology of primary biliary cirrhosis.

3. T-cells specific for the whole PDC and its E1 component are seen in significant numbers of normal control subjects as well as patients with primary biliary cirrhosis. Retention of potentially autoreactive cells in the normal T-cell repertoire has been reported for a number of other autoantigens.

4. T-cell epitopes appear to be widely distributed throughout PDC-E2. This is in contrast to the B-cell epitopes which are highly restricted to the inner lipoyl binding domain of the protein.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease which typically affects middle-aged women [1]. The early clinical features, of pruritus and, subsequently, obstructive jaundice, reflect the damage to the biliary epithelial cells lining the small intrahepatic bile ducts which is typical of PBC and which leads, ultimately, to bile duct destruction [2]. Bile duct damage is initially accompanied by granuloma formation and a portal tract mononuclear cell infiltrate. As the disease progresses duct loss is accompanied by ductular proliferation. Chronic inflammation and damage lead to progressive fibrosis with the eventual development of cirrhosis and its associated complications.

AUTOIMMUNE BASIS OF PBC

There is considerable evidence to suggest that PBC has an autoimmune aetiology [3]. Firstly, other autoimmune conditions, in particular autoimmune thyroid disease, are common both in PBC patients and their families, an associated risk which we have postulated recently as reflecting the possession of an 'autoimmune phenotype' [4]. Secondly, there is a human histocompatibility leucocyte antigen (HLA) association in PBC, albeit weak, with HLA-DR8 [5]. Finally, and most persuasively, serum autoantibodies are seen almost universally in affected patients. These autoantibodies are characteristically directed against a family of antigens, christened the M2 antigens, located on the inner mitochondrial membrane [3]. The dominant M2 antigen was cloned in 1987 using an expression vector system [6], and was subsequently identified as the dihydrolipoamide acetyltransferase (E2) component of pyruvate dehydrogenase complex (PDC) [7]. Autoantibodies to this antigen are found in the serum of over 90% of PBC patients and form an important diagnostic test for the condition [3]. Other autoantibodies, unique to PBC, were found to be specific for the E1α and E1β subunits of PDC, and the E2 subunits of the related oxoglutarate dehydrogenase complex and branched-chain oxo acid dehydrogenase complex [8, 9].

Key words: autoantigen, autoimmunity, biliary cirrhosis, liver, T-lymphocytes, tolerance.
Abbreviations: CLD, chronic liver disease; HLA, histocompatibility leucocyte antigen; NS, not significant; PBC, primary biliary cirrhosis; PDC, pyruvate dehydrogenase complex; PPD, purified protein derivative; SI, stimulation index; Th-1, T-helper 1; Th-2, T-helper 2.
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ROLE OF T-CELLS IN PBC

Although the PBC-specific anti-M2 autoantibodies have been well characterized [3], the precise role that they play, if any, in biliary epithelial cell damage is unclear. Immunohistochemical studies [10], and fluorescence-activated cell sorter analysis of mononuclear cells isolated from PBC liver [11], suggest that the mononuclear cell infiltrate in the portal tracts in the early stages of PBC, when biliary epithelial cell damage is occurring, consists predominantly of activated CD4+ and CD8+ α/β+ T-lymphocytes. Moreover, damage to the biliary epithelial cells is accompanied by upregulation of cell surface markers, such as class II MHC and intercellular adhesion molecule-1 (ICAM-1), which are typically induced by T-cell cytokines, a finding which suggests local T-cell activation [12]. Taken together, these findings suggest that T-cell-mediated mechanisms have an important role to play in the immunological damage to the biliary epithelial cells in PBC. However, in marked contrast to the autoantibody response, the autoreactive T-cell response in PBC had, until recently, remained poorly characterized.

Before the first identification of putative PBC-specific autoantigens in the late 1980s, studies of T-cell function in PBC had been limited to cell phenotyping [10, 13], characterization of responses to control antigens and mitogens [14–18], and the study of 'T-suppressor cell' function in vitro [19]. Peripheral blood- and liver-derived mononuclear cell phenotyping studies gave rise to often contradictory results, with the true significance of changes in the relative proportions and absolute numbers of different mononuclear cell types remaining far from clear [13]. A broad pattern did emerge of decreased T-cell numbers in the peripheral circulation, perhaps as a result of pooling in affected liver tissue, although these changes may simply have been a result of chronic liver disease rather than a feature of PBC per se. In vitro studies of response to mitogens, and in vivo and in vitro assessment of response to recall antigens, appeared to show impaired T-cell responses in PBC patients. In vivo cutaneous anergy was demonstrated to both tuberculin-purified protein derivative (PPD) and a chemical antigen, dinitrochlorobenzene, suggesting an immune response (in terms of our modern understanding of the immune response) defects in the pathway of T-helper 1 (Th-1) cell help for macrophage activation [14]. Defective in vitro mononuclear cell proliferative responses to mitogens such as phytohaemagglutinin [15] were demonstrated, suggesting a lowering in T-cell proliferative potential. Extension of these studies to a physiologically relevant recall antigen (PPD, as used in the studies of cutaneous delayed type hypersensitivity response by Fox et al. [15]) again showed an impaired proliferative response [16]. We have subsequently repeated some of these experiments, in particular looking at in vitro responses to both mitogens and recall antigens [17, 18]. Significantly, in these much larger repeat studies, we were unable to reproduce the findings of impaired proliferative responses to both phytohaemagglutinin and tuberculin-PPD in PBC patients, casting doubt on the original findings.

Two further experimental systems were used to study T-cell function (in an antigen non-specific manner). The first approach was to measure the ability of T-cells from PBC patients to help or suppress pokeweed mitogen-induced immunoglobulin synthesis by B-cells [19]. The second experimental system, used by the same investigators, was the autologous mixed lymphocyte reaction. Comparison of autologous mixed lymphocyte reaction (performed by measuring the proliferative responses of extensively washed T-cells co-cultured with autologous irradiated non-T-cells) between PBC patients and control subjects showed a 10-fold lower proliferation in PBC patients than control subjects. These studies appeared to show defective T-cell function in PBC, and the authors concluded that the defect in the T-cell response in PBC lay mainly in 'suppressor cell' function [19]. Experimental approaches of this type have fallen from favour in the study of autoimmunity in recent years, and it is difficult to interpret the findings in the light of our current understanding of the immune system.

T-CELL RESPONSES TO BOVINE PDC

The first identification of PDC as a putative B-cell autoantigen allowed a change of approach in the study of T-cell function in PBC by enabling the characterization of autoantigen-specific T-cell responses. Thus, instead of studying immune phenomena of the type described above which were of uncertain significance, we were able to characterize directly the T-cell responses which we felt played a significant role in biliary epithelial cell damage in PBC. We therefore set out to study T-cell responses to PDC and its components in PBC. Using a biochemical approach, differential polyethylene glycol precipitation, we purified whole PDC from heart muscle. The whole complex was then broken down to its individual components: PDC-E1 (pyruvate dehydrogenase), PDC-E2 (dihydrolipoamide acyltransferase) and protein X which co-purify, and PDC-E3 (dihydrolipoamide dehydrogenase) which were separated by gel filtration and ultracentrifugation. This purification approach gave rise to enzymically active whole PDC (as assayed in terms of the reduction of NAD+ to NADH). Moreover, the separated component subunits, when added together, rapidly recombined to form enzymically active complex. The fact that the enzyme antigen remained structurally and functionally intact represented one of the advantages of using biochemically purified native antigen, as opposed to recombinant antigen or synthetic peptides, in the study of autoreactive T-cell responses.
Peripheral blood T-cell primary proliferative responses to each of the antigens were measured in a series of 24 PBC patients (15 with precirrhotic liver disease, nine with cirrhosis), 20 normal control subjects and 28 subjects with other forms of chronic liver disease (CLD) [20]. The subject groups were matched for age and sex. Six-day primary proliferation assays were performed for PDC, PDC-E1, PDC-E2/X and PDC-E3 at a range of concentrations, T-cell proliferative responses being assayed by $[^{3}H]$thymidine incorporation. Intriguingly, positive T-cell responses to PDC [Stimulation index (SI, defined as the ratio of the mean incorporated counts in response to antigen to the mean count in control wells) $>2.5$] were seen in a majority of patients in both control groups as well as in the PBC patient group, with no significant differences between the groups with respect to either the frequency or the magnitude of positive responses or the antigen concentration giving maximal response. Control mean incorporated c.p.m. were similar in all three subject groups (normal $829 \pm 580$, CLD $664 \pm 593$, PBC $483 \pm 319$). Mean SI for the PBC patients was 7.78, compared with 6.36 for all control subjects [$P$ not significant (NS)]. Twelve out of 24 (50.0%) PBC patients had a positive response to PDC compared with 10/20 (50.0%) normal control subjects and 14/28 CLD (50.0%) patients ($P$ = NS). When the two control groups were compared, the mean SI for normal subjects was 6.41 compared with 6.32 for the CLD patients ($P$ = NS). The frequency of significant responses to PDC was higher in precirrhotic than in cirrhotic patients (10/15 compared with 2/9), although this difference did not reach statistical significance. No significant difference was seen between cirrhotic and non-cirrhotic CLD control subjects.

When responses were measured to PDC-E1 it became clear that a significant proportion of the response to the whole complex was directed against this subunit. Significant responses to PDC-E1 were once again seen in both PBC patients and control subjects. These responses showed a high correlation to those seen in both groups to whole PDC (control subjects $P$ < 0.0001; PBC patients $P$ = 0.0015). There were no significant differences in responses between PBC patients and the whole control group (PBC mean SI 7.33, control mean SI 4.73, $P$ = NS; PBC 15/24 positive, control subjects 25/48 positive), or between the two control groups (normal mean SI 4.17, CLD mean SI 5.16, $P$ = NS; normal 11/20 positive, CLD 14/28 positive). As with the response to PDC, PDC-E1 concentrations giving rise to maximal T-cell response were similar in all subject groups.

In contrast to PDC-E1, T-cell responses to PDC-E2/X were highly restricted to PBC patients. Significantly greater responses to PDC-E2/X were seen in PBC patients than in control subjects. The mean SI was significantly higher in the PBC patients (4.91 compared with 1.33, $P$ < 0.0001), as was the proportion with a positive response (14/24 compared with 6/48, $P$ < 0.0001). Within the control group no significant difference was seen between the normal subjects and the CLD patients (normal 1.31, CLD 1.35, $P$ = NS; normal 3/20 positive, CLD 3/28 positive). Positive responses to PDC-E2/X were significantly more frequent in precirrhotic (12/15) than in cirrhotic (2/9, $P$ < 0.05) patients. Fluorescence-activated cell sorter analysis has subsequently been used to confirm that the responding cells are CD4+ T-cells. Responses to PDC-E3 were equally uncommon in all subject groups.

These studies suggested that peripheral blood T-cell responses to bovine PDC-E2/X are strongly associated with PBC. In particular, responses are strongest and most frequently seen in the early, immunologically active, stages of the disease when bile duct damage occurs. The early-stage predominance of the T-cell responses is in contrast to the serum anti-PDC-E2/X titres which rise as disease progression occurs [21]. Although the T-cell response to PDC-E2/X in PBC patients showed no correlation with biochemical or haematological disease parameters, one interesting clinical correlation was seen. If the PBC patient group was subdivided according to the presence or absence of other autoantibodies a clear pattern was seen. Patients found to have other, non-M2, serum autoantibodies (with anti-smooth muscle antibodies excluded) were found to have significantly stronger PDC-E2/X-specific T-cell responses than subjects without other autoantibodies (mean SI 8.3 ± 7.1, 8/8 positive with other autoantibodies; mean SI 4.9 ± 5.5, 6/12 positive without other autoantibodies). Strikingly, the group with anti-smooth muscle antibodies showed no response to PDC-E2/X (mean SI 0.9 ± 0.3, 0/4 positive). These preliminary findings raise the possibility that the clinical syndrome recognized as PBC represents a final common pathway of tissue damage, with immune responses of different specificities, only one of which is directed against PDC-E2/X, giving rise to bile duct damage. This is in keeping with the observation that graft-versus-host disease, which results from allogeneic T-cell activation, induces biliary epithelial damage reminiscent of that seen in PBC.

Two other studies have looked at T-cell responses to bovine PDC and its subunits in PBC, with differing findings [22, 23]. Lohr et al. [22] demonstrated positive peripheral blood T-cell proliferative responses to whole bovine PDC in 11/15 PBC patients, with a similar response magnitude to that reported in our study [20]. In contrast to our study, no positive responses to bovine PDC were seen in chronic liver disease control subjects ($n$ = 20) or normal subjects ($n$ = 5). When T-cell clones derived from PBC patients' liver were characterized with respect to antigen specificity, 9/115 showed class II-restricted PDC specificity. None of these clones,
however, showed a positive response to bovine PDC-E2/X. It is possible that the differences between these two studies result from variations in the degree of purity of the antigen preparations used. Van de Water et al. [23] only demonstrated positive peripheral blood T-cell proliferative responses to whole bovine PDC in 1/19 PBC patients. They did, paradoxically, see responses to PDC-E2/X in 10/19 PBC patients and to PDC-E1 in 7/19. As in our larger study responses to PDC-E2/X were PBC-specific; responses to PDC-E1 and the whole complex, however, were not seen in 12 control subjects.

**T-CELL RESPONSES TO HUMAN PDC**

The major concern in ascribing significance to these PDC-E2/X-specific T-cell responses was that, in all these studies, the antigen used was of bovine origin, and was therefore a xenoantigen. Although PDC-E2 is highly conserved across mammalian species (413 out of 460 residues are identical in human and rat PDC-E2) it was impossible to exclude, on the basis of these studies alone, the possibility that the responses being measured were xenoantigenic in nature. In order to exclude this possibility we went on to purify human PDC-E2 from heart muscle [24] and to study T-cell responses to it in PBC patients [25]. This study was unique as it represented one of the first occasions in which T-cell responses were measured to a tissue-derived intact native human autoantigen in a human autoimmune disease. Previous studies of T-cell specificity in human autoimmune diseases have largely, because of difficulties in purifying adequate amounts of native human antigen, relied on the use of either non-human equivalent antigens (for example bovine PDC in our early studies in PBC and Torpedo acetylcholine receptor in the study of myasthenia gravis), recombinant antigens over-expressed in prokaryotes or sequence-specific peptides. Each of these alternative approaches has theoretical and practical disadvantages. Through the use of native human PDC-E2/X we were able to confirm that T-cell responses to this antigen are indeed uniquely associated with PBC, significant T-cell proliferative responses to human PDC-E2/X being seen in a majority of PBC patients (15/28), but in only a small minority of normal and chronic liver disease control subjects (5/32, \( P = 0.003 \)).

As with bovine antigen, when T-cell responses to human PDC and PDC-E1 were measured, a pattern of equal and significant response in both PBC patients and control subjects was once again seen. The findings from this study therefore confirmed our previous observations that T-cells responsive to PDC and its PDC-E1 subunit are present in a significant proportion of the normal population. This is in contrast to the PBC-restricted PDC-E2/X responses. The retention in the normal peripheral repertoire of autoreactive T-cells specific for several autoantigens has been reported previously [26], emphasizing the important role played by peripheral mechanisms in the control of T-cell self-reactivity. This phenomenon of controlled retention of self-reactive T-cells has been suggested to have a physiological role, providing a preformed response potential to microbial antigens with a human equivalent [26]. The observation that patients with acute mycobacterial infections mount an acute, and self-limiting, anti-PDC response which presumably aids mycobacterial clearance provides evidence in support of this hypothesis [27].

No other studies of T-response patterns to native human PDC-E2/X in PBC have been carried out. Responses have been studied, however, to recombinant human PDC-E2 [23, 28], and human PDC-E2 sequence-specific peptides [29]. These studies, although highlighting some of the problems inherent in using recombinant antigen and sequence-specific peptides, confirm the PBC restriction of anti-PDC-E2/X T-cell responses.

**AUTOEPITOPE LOCALIZATION WITHIN PDC-E2**

If, as we suggest, PDC-E2 is an important T-cell autoantigen in PBC, where within the molecule are the important T-cell autoepitopes? Several studies, including one performed by our group, have set out to map the significant T-cell autoepitopes within PDC-E2 [23, 29, 30]. PDC-E2 consists of several highly conserved functional domains, inner and outer lipoyl acid binding domains, an E1/E3 binding domain and a catalytic domain, all linked together by flexible linker sequences. The anti-PDC-E2 autoantibody response characteristic of PBC is highly specific for the inner lipoyl domain [31]. Van de Water et al. [23, 28] studied the specificities of PBC liver-derived T-cell clones and peripheral blood mononuclear cells. They demonstrated lower responses to a recombinant protein containing only the inner and outer lipoyl domains of PDC-E2 than to whole molecule (of bovine origin). This finding appeared to suggest that there are T-cell epitopes in both the lipoyl domains and in the non-lipoyl portions of PDC-E2. It is important to note, however, that in this study comparisons were being drawn between responses to a tissue-derived xenoantigen and a recombinant human polypeptide. Differences in response could be accounted for wholly by differences in protein packaging and in processing by antigen-presenting cells. The possibility that such artefactual differences in antigen handling may lead to apparent differences in antigenicity is highlighted by the finding, in this study, that individual clones responsive to a shortened recombinant containing only the inner lipoyl domain did not respond to the whole inner and outer lipoyl domain recombinant protein, a construct which contains the inner lipoyl domain in its entirety.

Shimoda et al. [29] used overlapping peptides synthesized from the sequence of human PDC-E2 to
study autoepitope distribution. Peripheral blood primary responses were seen to peptide pools scanning the inner lipoyl domain, the outer lipoyl domain and the catalytic domain (but not the leader sequence and the flexible linkers), once again suggesting that T-cell epitopes, in contrast to the highly restricted B-cell epitopes, are widely distributed within PDC-E2. Subsequent cloning experiments were only successful in deriving clones specific for the inner lipoyl domain pool (although this may reflect enhanced B-cell presentation by cells with surface immunoglobulin highly restricted for the inner lipoyl domain). We used a different, biochemical, approach to address this question, using limited trypsic digestion to cleave human PDC-E2 into a catalytic domain and a greater lipoyl domain containing both the inner and outer lipoyl domains. The individual domains, purified by gel filtration, were used in peripheral blood primary T-cell proliferation assays in a series of PBC patients [31]. Those patients responsive to the whole PDC-E2 molecule showed, almost without exception, responses to both the lipoyl and catalytic domain. Those patients showing no response to whole PDC-E2 showed no response to either subdomain. These findings confirmed that T-cell epitopes are widely distributed in PDC-E2. The absence of response to the subdomains when no response was seen to whole PDC-E2 suggested that cryptic epitopes are not revealed by the trypsic cleavage of PDC-E2.

**BREAKDOWN OF SELF-TOLERANCE IN PBC**

Based on these studies it is our hypothesis that T-cell responses to PDC-E2/X play an important role in the immunopathology of PBC. This raises two important questions. Firstly, how does breakdown of tolerance to such a ubiquitous and evolutionarily conserved antigen occur, and secondly, why does tissue damage occur in such an organ-specific manner if the antigen is universal within the body? At present neither question can be answered, but it is possible to speculate using available experimental data. Experimental evidence can be interpreted as supporting three potential mechanisms of breakdown of self-tolerance to PDC-E2/X in PBC.

**Molecular mimicry**

**Bacterial cross-reactivity model.** Several pieces of evidence have been put forward to support molecular mimicry as an aetiological mechanism in PBC. This suggestion pre-dated the identification of the mitochondrial autoantigens in 1988. The first observation that PBC patients' sera cross-react with bacterial antigens was made in 1976 when Sayers et al. [32] demonstrated cross-reactivity with *Para- coccius denitrificans*. Subsequently, cross-reactivity was demonstrated with a number of other bacteria, in particular with rough mutants of the Enterobacteriacea, which have abnormal cell surface expression of antigens cross-reactive with the PBC-specific autoantigens, and which are present in increased numbers in the stools of PBC patients. This high degree of cross-reactivity with bacterial antigens led to the suggestion that molecular mimicry between bacterial and self-antigens may have a role to play in the pathogenesis of PBC [33].

Further evidence supporting an aetiological role for bacteria in PBC came from the finding that PBC patients have an increased incidence of urinary tract infections with Gram-negative bacteria, with a high proportion of the organisms in infected urine being rough mutants. Intriguingly, when a group of non-PBC patients with recurrent urinary tract infections were studied, a similarly high proportion of rough mutants was noted. A significant proportion of this group (69%) was found to have a low titre of anti-M2 antibodies in their serum on immunoblotting. Although the significance of the high incidence of urinary tract infections in PBC patients is not clear, antigen presentation via the urinary tract (part, as is the biliary tree, of the mucosal immune system) could explain the mucosal tropism of PBC.

The explanation for these apparently highly significant cross-reactions with bacterial antigens came, of course, with the identification of PDC-E2, a protein highly conserved in evolution, as the dominant M2 antigen. Far from immunological cross-reactivity between bacterial and human PDC-E2 being a novel or unexpected finding, many of the early experiments aimed at characterizing the B-cell epitopes within E2 utilized *Escherichia coli* PDC-E2, tacitly relying on this cross-reactivity occurring [3]. In view of the identification of PDC-E2 as the dominant autoantigen the question of molecular mimicry in PBC must be viewed in a different light. Molecular mimicry models of autoimmunity suggest that cross-reactivity between self-antigens and unrelated (but structurally similar) microbial antigens leads to a breakdown in self-tolerance (an example being a potential cross-reactivity between the nuclear antigen gp-210 and the *E. coli* mutY gene product). The molecular mimicry models put forward in PBC, based on these bacterial infection studies, are suggesting that it is a response to a structurally almost identical bacterial equivalent of a human protein that is responsible for the cross-reactive response. This raises the obvious question why, if PDC with a high degree of similarity to the human equivalent is almost ubiquitous within bacterial species which in turn universally colonise humans, are pathological autoreactive responses to this antigen so infrequent. The evidence regarding immunogenetic susceptibility to PBC does not suggest that there is a highly restricted genotype in PBC patients which might allow a common immunological trigger to give rise to a highly infrequent pathological response.

The debate regarding a bacterial trigger playing a role, through molecular mimicry, in the pathogenesis of PBC has reopened recently with the observa-
tion that PBC patients have antibodies in their serum which cross-react with antigens derived from the atypical mycobacterium M. gordonae [34]. Unsurprisingly, these cross-reactive antibodies were demonstrated to be specific for the members of the 2-oxoacid dehydrogenase family of multi-enzyme complexes. These findings, which were not reproducible in a subsequent study, represent a further example of cross-reactivity between bacterial and human PDC, with the attendant questions regarding relevance.

MHC class II molecular mimicry model. More recently, a second model of molecular mimicry has been put forward by Burroughs et al. [35]. Sequence comparison between mammalian and prokaryotic PDC-E2, and the α-chain of HLA-DR shows some similarity between the inner lipoic domain of PDC-E2 and the region between residues 80 and 97 of HLA-DR-α. This similarity is limited (7/17 residues comparing E. coli PDC-E2 and HLA-DR-α), and is associated with significant differences in the tertiary structure of the respective proteins in their relevant regions (PDC-E2 lipoic region has a turn between β-strands, HLA-DR-α an α-helix). This sequence similarity, taken with the observations that the equivalent peptide from the mouse class II MHC I-E α-chain is bound to, and presented by, I-A (the mouse equivalent of HLA-DQ), and that MHC class II expression is significantly upregulated on biliary epithelial cells, has led to the following hypothesis. Infection with bacteria, possibly of the types identified above as being particularly prevalent in PBC, leads to T-cell responses specific for bacterial PDC-E2 inner lipoic domain. These T-cells are then locally stimulated by the cross-reactive HLA-DR-derived epitope presented by HLA-DQ expressed on the surface of biliary epithelial cells, leading to cell damage. There are, however, two significant flaws in this hypothesis. Firstly, the degree of similarity between HLA-DR α-chain and PDC-E2 is relatively low, particularly in terms of T-cell epitopes where single residue changes can lead to significant changes in T-cell specificity. Secondly, abnormal expression of class II on the surface of the biliary epithelial cells would have to occur before the development of PBC. Most studies of class II expression suggest that it in fact occurs as a result of the inflammatory response in the portal tracts. Expression before the development of the PBC inflammatory response would therefore require a separate pathological process to occur in the pre-PBC phase.

Abnormal self-antigen presentation

A second hypothesis to explain the breakdown of self-tolerance in PBC is the cryptic self, or abnormal self-antigen presentation hypothesis. Although PDC is present in all cells in the body it is normally sequestered in the mitochondria. After nuclear transcription and translation the protein is targeted to, and actively transported into, the inner mitochondrial space, with no cell surface expression being seen under normal conditions. The majority of cells expressing PDC (including the biliary epithelial cells) additionally do not normally express MHC class II on their surface. The mitochondrial location of the complex, and its resulting exclusion from both the class I and class II antigen processing pathways, leads to it being 'hidden' from the immune system. Even in those cells expressing MHC class II on their surface, the trans-cytoplasmic transport phase of the life span of PDC would not render it susceptible to class II presentation. There is increasing evidence to suggest that aberrant biliary epithelial cell surface expression of PDC-E2, or a molecule immunologically cross-reactive with it, is seen in PBC [36]. Theoretically, this abnormal expression of PDC-E2 on the biliary epithelial cell surface may allow it to aberrantly enter the class II processing and antigen presentation pathway. The concomitant upregulation of class II in PBC leading to high level class II presentation of PDC-E2-derived peptides could therefore, in principal, lead to autoreactive T-cell stimulation and a breakdown of tolerance to self. The failure of biliary epithelial cells to express important co-stimulatory molecules such as B7-1 and B7-2, even after cytokine stimulation [12], provides significant evidence against this hypothesis.

Defective T-cell control

As discussed above, significant proportions of patients with both recurrent urinary tract infections and acute tuberculosis have anti-PDC-E2/X antibodies in their serum. In the latter case at least the antibody responses have been shown to be short-lived and self-limiting. In both cases there is no evidence whatsoever to suggest that the affected subjects are at increased risk of developing PBC. These findings suggest that, far from being 'forbidden', anti-PDC-E2/X responses can be mounted at physiologically appropriate times, and may indeed, as a result of the high degree of sequence conservation between different bacterial species, represent important protective responses against bacterial infection. Indeed, were the potential to mount anti-self-PDC-E2/X responses to be retained in the normal repertoire, then autoreactive PDC-E2/X-specific T-cells would form a ready-made defensive population which could be reactivated in response to bacterial PDC-E2/X in the immunologically correct context, leading to a rapid antibacterial response. Based on this evidence it could therefore be argued that the presence of anti-PDC-E2/X responses in PBC represents not a breakdown of tolerance leading to the retention of autoreactivity, but a failure of control leading to expression in the wrong context.

If potentially autoreactive PDC-E2/X-specific T-cells are retained in the peripheral repertoire, then peripheral control must be exerted under
normal conditions (including in the in vitro setting). The extremely low incidence of pathological anti-PDC-E2/X responses in the general population suggests that these control mechanisms are normally highly effective. One important mechanism of peripheral control appears to be through the reciprocal control of different T-cell subsets [37]. Recent interest has focused on the role played by interactions of the Th-1 and T-helper 2 (Th-2) CD4+ T-cell subsets [38]. Th-1 cells secrete interferon-γ and interleukin-2, and assist macrophage activation and cytotoxic T-cell function. Th-2 cells secrete interleukin-4 and interleukin-10 and assist the production of mature antibody responses. One important mechanism of peripheral T-cell tolerance appears to be control of autoreactive Th-1 cells, retained in the normal repertoire, by Th-2-secreted cytokines [37]. Factors leading to a disturbance of this controlling balance, including perhaps localized infection or superantigen activity, possibly on a background of genetic susceptibility [4], could give rise to aberrant expression of normally controlled autoreactivity. Preliminary experiments looking at cytokine secretion patterns in response to antigens in PBC seem to demonstrate abnormal cytokine levels in comparison to normal control subjects [39]. Further work is required to see whether this may help to explain the breakdown of self-tolerance seen in PBC.

CONCLUSIONS

Since the initial identification of putative autoantigens in PBC in 1988 we have gone a considerable way towards understanding the immunopathology of this condition. This is important for two reasons. Firstly, PBC presents a fascinating model in which to study autoimmunity in humans. The autoantigens have been identified, and, importantly, can be purified in human form allowing the study of truly autoreactive T-cell and B-cell responses. Moreover, affected liver tissue is available to study in both early (diagnostic liver biopsy material) and late ( explanted organ at liver transplantation) disease stages. Secondly, and more importantly, PBC is a condition for which currently available treatments are extremely poor at preventing disease progression [40]. This therapeutic poverty is reflected in the fact that PBC represents one of the commonest indications for liver transplantation in the U.K. Our increasingly advanced understanding of the immunopathology of the condition, together with the ability to diagnose it in its earliest, even pre-disease, stages raises the real possibility that immunotherapy may in the future allow us to improve this currently bleak prognosis.

REFERENCES


