



Review

The Immunopathology of Primary Biliary Cirrhosis: Thoughts for the Millennium

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Abstract. Primary biliary cirrhosis is an organ specific autoimmune disease that produces progressive cholestatic liver failure. It is predominantly a disease of women characterized by chronic progressive destruction of small intrahepatic bile ducts with portal inflammation and ultimately fibrosis. The serologic hallmark of primary biliary cirrhosis (PBC) is the presence of antibodies to mitochondria. The mechanisms by which and if which such antibodies produce liver tissue injury is unknown. However, the presence of these antibodies have allowed detailed immunological definition of the antigenic epitopes, the nature of reacting autoantibodies and the characterization of T cell responses. Several mechanisms may now be proposed regarding the immune mediated bile duct damage in PBC, including the possible role of T cell-mediated cytotoxicity and intracellular interaction between the IgA class of antimitochondrial antibodies (AMA) and mitochondrial autoantigens. The advent of molecular biology, the ability to clone and define epitopes, and the use of *in situ* nucleic acid hybridization, have all led to advances in understanding the natural history of immunopathology in PBC. There are major questions which remain unanswered, including, of course, etiology, but also including the questions of why there is female predominance, the absence of PBC in children, the relative ineffectiveness of immunosuppressive drugs, and the specific role of mitochondrial antigens. In this review, we focus on these issues and particularly on the immunobiology of patients with this disease.

Key words: primary biliary cirrhosis; autoimmunity; mitochondria; pyruvate dehydrogenase.

Primary biliary cirrhosis (PBC) is a chronic, progressive cholestatic liver disease of unknown etiology. It usually affects middle-aged women, and often leads to liver failure and the need for liver transplantation^{34, 53}. Histopathologically, PBC is characterized by chronic progressive destruction of small intrahepatic bile ducts with portal inflammation, leading initially to fibrosis

and later to cirrhosis^{51, 52, 62}. PBC is considered to be an organ-specific autoimmune disease based on the close association between PBC and antimitochondrial antibodies (AMA)^{9, 14, 17, 18, 67, 74}. A major advance in defining the pathogenesis of PBC made during the last decade by the precise identification of the mitochondrial proteins that are the targets of such autoantibodies.

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However, the mechanisms by which such antibodies induce liver tissue injury and disease remain to be defined and are the focus of investigation in several laboratories including our own. Our studies are addressing several questions: Firstly, what is the precise cause or first event of PBC? The presence of a detectable AMA might be a epiphenomenon that appears after some other triggering event. Secondly, why is this autoimmune response directed against such highly conserved series of mitochondrial antigens and why does such response induce damage to a highly focused minor population of BECs (BEC)? Thirdly, how is this autoimmune response initiated against “mitochondria” which are shielded by their intracellular location?

Autoantibodies in PBC

AMA

The targets of AMA are members of the 2-oxoacid dehydrogenase complex (2-OADC), including the E2 subunit of pyruvate dehydrogenase complex (PDC-E2), the E2 subunit of branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), the E2 subunit of 2-oxoglutarate dehydrogenase complex (OGDC-E2), and the dihydrolipoamide dehydrogenase binding protein (E3BP) (Table 1)^{9, 14, 17, 18, 67, 74}. The most common reactivity of AMA-containing sera from PBC patients is against PDC-E2. While some patients have AMA that react with PDC-E2 alone, most patients also show reactivity against OGDC-E2 or BCOADC-E2. Reactivity against BCOADC-E2 or OGDC-E2 alone is less common. These target antigens are located in the mitochondrial matrix, associated with the inner membrane, and catalyze the oxidative decarboxylation of various α -keto-acid substrates^{5, 23, 81}.

The E2 enzymes have a common structure which consists of the N-terminal domain containing the lipoyl group(s), the peripheral subunit binding domain respon-

sible, at least in part, for binding the E1 and E3 components together; and the C-terminal inner core houses the active site responsible for the acetyltransferase activity. Several studies using oligopeptides or recombinant proteins have shown that the predominant epitope of PDC-E2 is located within the lipoyl domain of PDC-E2^{66, 74}.

AMA also reacts with the outer domain, but at a 100-fold lower dilution, and only one in 26 PBC sera reacts weakly to the E1/E3 binding region⁶⁶. The mapping of B cell epitopes using truncated constructs and the combination of peptides reveals that the reactive AMA to PDC-E2, BCOADC-E2, and OGDC-E2, each recognizes a conformational epitope including the inner lipoyl domain^{6, 41, 50, 66}. These domains contain amino acids motif ETDKA, ETDK(T), (GlnS) DKA with lipoyl acid covalently bound to the ϵ group of lysine (K).

The highly conserved structure in the E2 subunit of 2-OADC and their lipoyl domains suggests that lipoic acid may be a part of the immunodominant epitope. Several studies have been conducted to address the contribution of lipoic acid to the reactivities of the autoantibodies^{13, 15, 42, 57}, and the results are somewhat contradictory. However, the data clearly show that AMA is capable of binding to both lipoylated and unlipoylated PDC-E2.

An additional target of some AMA is the E1 α subunit of PDC (PDC-E1 α) which lacks the lipoyl domain (Table 1)¹⁶. Its autoepitope of interest is located at the phosphorylation and TPP-binding site²⁸. Interestingly, AMA, especially of the IgA isotype, and the autoantigens, PDC-E2, OGDC-E2, and BCOADC-E2 have all been readily detected in the bile of patients with PBC⁵⁵.

Autoantibodies Other Than AMA

Although AMA are considered the hallmark diagnostic feature of PBC, they are not the only disease-specific autoantibodies; a number of additional auto-

Table 1. Characteristics of antimitochondrial autoantibodies (AMA) and mitochondrial antigens in PBC

Antigen	Molecular weight (kDa) ¹	Frequency (%) in PBC ²	Lipoyl domain	B cell epitopes	Major Ig isotype	Inhibition of function by AMA
PDC-E2	74	95	+	outer and inner lipoyl domain	IgG3, IgM	+
BCOADC-E2	52	53–55	+	lipoyl domain	NR	+
OGDC-E2	48	39–88	+	lipoyl domain	IgG2, IgM	+
PDC-E1 α	41	41–66	–	TPP binding and phosphorylation sites	NR	+
E3BP	55	95	+	NR	NR	NR

¹ Determined by SDS-PAGE and immunoblotting against PBC sera.

² Determined by immunoblotting or ELISA against recombinant proteins.

NR – not reported.

antigens have been identified within the nuclear membrane. These autoantigens include the nuclear pore protein, gp210, which is a 210 kDa transmembrane glycoprotein believed to be involved in the attachment of pore complex constituents within the nuclear membrane. Antibodies directed against gp210 are found in about 25% (range, 10 and 40%) of patients with AMA-positive PBC and up to 50% of those with AMA-negative PBC. The disease specificity for the detection of such autoantibodies by immunoblotting is over 99%⁴. In contrast to AMA, antibodies against gp210 have prognostic significance²⁷. Other autoantigens include the nuclear pore protein, p62, which is a nuclear pore glycoprotein specifically recognized by PBC sera and present in about 25% of patients with PBC⁷⁹. The presence of anti-gp210 and p62 in PBC sera appear to be mutually exclusive. Finally, a minority (< 1%) of PBC sera shows reactivity against the inner nuclear membrane protein, lamin B receptor. Lamin B receptor (LBR) is an inner nuclear membrane protein that binds nuclear lamins and double-stranded DNA; LBR may play a role in anchoring nuclear lamins and heterochromatin during interphase. Although < 1% of PBC sera contain antibodies to LBR, there does appear to be disease specificity for such autoreactivity^{4, 54}.

The Characteristics of T Cells in Primary Biliary Cirrhosis

Phenotypes of T cells in PBC

The presence of T cell infiltrates within the portal tract had led to much speculation concerning the role of these cells in the pathogenesis of PBC. CD3⁺, CD4⁺, and CD8⁺ T lymphocytes expressing the T cell receptor (TCR) $\alpha\beta$ chain are the predominant cell types in and around the portal areas in PBC³⁷. The CD4/CD8 ratio has been reported to range from 2 to 2.5: 1^{8, 24}. CD8⁺ T cells are most abundant during the early stages of the disease and may be involved in cytotoxic bile duct injury. As the disease progresses, a higher proportion of CD4⁺ T cells is observed^{26, 37}. Less than 10% of the T cells express the TCR $\gamma\delta$ chains³⁷.

T cell epitope in PBC

A study by VAN DE WATER et al.⁷³ demonstrated for the first time that T cell clones reacting with PDC-E2 and/or BCOADC-E2 were present in the liver biopsy specimens of patients with PBC. These clones were CD4⁺, TCR $\alpha\beta$ ⁺ and produced IL-2 specifically in re-

sponse to PDC-E2 or BCOADC-E2⁷³. The precise T cell autoepitopes of PDC-E2 have been characterized by SHIMODA et al^{64, 65}. During the course of these studies, a number of PDC-E2 specific T cell clones from not only the peripheral blood, but also from the explanted livers and regional lymph nodes (RLH) of patients with PBC was established. Using a battery of overlapping peptides covering the full length PDC-E2 molecule, the minimal autoepitopes recognized by these T cell clones was defined and found to locate within the same region of PDC-E2 peptide 163–176 (GDLLAEIETDKATI), which is contained within the inner lipoyl domain of human PDC-E2⁶⁴. Additional peptide specificity studies revealed a degree of cross-reactivity for the T cell clones with specificity for the PDC-E2 peptide 163–176. Thus, such PDC-E2 163–176 were also shown to proliferate with PDC-E2 peptide 36–49 and OGDC peptide 100–113. A fine analysis of the peptides that react with such T cell clones led to the identification of a common T cell epitope motif ExETDK^{64, 65}. These data provide evidence for a major role for the PDC-E2 peptide 163–176 and/or peptides bearing a similar motif in the pathogenesis of PBC. Interestingly, autoreactive T cells and autoantibodies from PBC patients both recognize the same dominant epitope. The importance of such T cell clones was highlighted by the finding that there is a disease-specific 100–150-fold increase in the precursor frequency of such PDC-E2 163–176-specific T cells in the hilar lymph nodes and liver when compared with concordant PBMC samples from the patients with PBC. In addition, in the early or moderate stage of PBC, the frequency of peripheral T cells, responding to peptide 163–176, is significantly higher than in end-stage PBC, although it is not currently known whether this represents the disappearance of cells during the course of the disease or a progressive homing of cells to the liver or the RLH⁶⁵.

MHC and T cell recognition in PBC

In Caucasians, PBC has been reported to be associated with HLA-DR3, HLA-DRw8, and possibly HLA-DR4. An association with HLA-DR2 and DP B1 0501 has been reported in Japanese patients with PBC¹². Thus far, no consensus exists regarding the association between PBC and HLA. Of interest was the finding that the T cell clones specific for human PDC-E2 163–176 were restricted by the MHC molecule: HLA DRB4 0101^{64, 65}. However, the frequency of this relatively less polymorphic HLA DRB4 0101 allele among patients with PBC is similar to control Japanese populations.

T cell receptor in PBC

Several TCR-V β usage studies have been performed on liver-derived T cells. Some studies showed that the T cell clones established from PBC patients had limited diversity and sequence analysis of CDR3 and revealed the presence of conserved residues, no random N additions, and a common motif within the CDR3^{49, 69}. However, another study showed that TCR-V β usage by PDC-reactive T cell clones infiltrating the liver was remarkably heterogeneous⁷². The reasons for such discrepant results are not clear. By the nature of techniques utilized, all the T cell clones established thus far express CD3⁺, CD4⁺, CD45RO, and TCR $\alpha\beta$ ⁺ denoting that each of these T cell clones is a memory helper T cell. The role, characteristics and functions of CD8⁺ cytotoxic T cell in PBC to date remain largely unknown. Clearly additional studies are required to define a role, if any, for CD8⁺ cytotoxic T cells in PBC.

Immunopathological Events Involved in PBC Liver

Histopathology of PBC

Chronic, progressive, non-suppurative cholangitis, involving small intrahepatic bile ducts, is the fundamental lesion in PBC^{51, 52, 62}. Interlobular bile ducts are damaged and eventually disappear from the liver in the advanced stages⁵¹. Progressive bile duct loss following chronic cholestasis and necroinflammatory processes of the hepatic parenchyma are thought to be responsible for progression of the disease to cirrhosis^{51, 62}. Other diseases, such as liver allograft rejection and chronic hepatitis C⁷⁸ may also show bile duct lesions similar to those of PBC and should be differentiated from PBC.

Inflammatory cells and cytokine profiles in PBC

The inflammatory infiltrates within the portal tract contain a predominance of TCR $\alpha\beta$ ⁺ CD3⁺ T cells which include both CD8⁺ and CD4⁺ subsets. CD4⁺ T cell lineages are more common near bile ducts, while CD8⁺/CD11b-negative cytotoxic T cells predominate in areas of piecemeal necrosis^{37, 24}. While the CD8⁺ T cell lineages are most abundant during the early stages, a higher proportion of CD4⁺ T cells is observed as the disease progresses. Macrophages make up about 30% of the cellular infiltrate⁸, and CD20⁺ or CD22⁺ B cells and cells bearing surface immunoglobulins ac-

count for about 10% of the population^{24, 37}. Eosinophils comprise an appreciable proportion of the inflammation⁶⁸. Natural killer (NK) cells (CD16, CD56, or CD57) account for approximately 5% of the cellular infiltrate. Interdigitating dendritic cell (DC) are found between BEC in PBC, often near breaks in the basement membrane and in the periductal granulomatous response^{11, 58}. The presence of DC and granulomas implies that antigen presentation is occurring and that, possibly, the inciting antigen(s) is persistent. It also suggests that a delayed-type hypersensitivity response is one possible effector mechanism, in which Th1-type cytokines IFN- γ , IL-2, and tumor necrosis factor α (TNF- α) play important roles.

Increases in IL-2, -5, and -6 and IFN- γ and TGF- β mRNA have been reported in liver tissues of patients with PBC⁴⁶. While this cytokine profile is not specific for PBC, it reflects the inflammatory microenvironment. However, there is a preferential up-regulation of IL-5 mRNA, which is a Th2-type cytokine gene, in comparison to autoimmune chronic hepatitis⁴⁶. In the later stages of the disorder, the small bile ducts are destroyed and the DC, granulomas, and inflammation usually subside^{11, 58}.

The prototype Th2 cytokines have been proposed to play a role in the establishment and persistence of inflammation in a variety of autoimmune disorders⁷. Although these findings suggest that PBC is associated with predominantly a Th2 cytokine, the frequency of IFN- γ (a predominant TH1 cytokine) expressed predominantly in association with damaged bile ducts, provides suggestive evidence that such a cytokine is likely associated with the development of CTLs²¹. The finding that PBC liver tissues are also associated with eosinophilic infiltrates, increased expression of IL-5, and in select areas IFN- γ as described above, it is likely that the select role of Th1 vs Th2 set of cytokines in the pathogenesis of PBC remains unclear. Furthermore, the high levels of IL-10 found in normal liver suggest that complex networks of cytokines will be involved in regulating the development of liver cell damage and chronic persistent inflammation.

Phenotypical changes of BECs in PBC

As the BECs are a major target of immune-mediated attack, a number of studies have focused on the study of the changes of BECs in PBC in comparison with other hepatobiliary diseases (Table 2). Because of the specificity of interlobular bile duct damage in PBC patients, it is logical to hypothesize that this particular segment of the bile ducts expresses a specific antigen

Table 2. Phenotypic changes to biliary epithelium of small bile ducts in PBC

	Normal	PBC	Other LD
Autoantigens			
PDC-E2	+	+++	+
PDC-E3BP	+	+++	+
Anion-exchanger-2	+	-/+	+
Immune adhesion molecules			
MHC class I	+	+	+
MHC-class II (HLA-DR)	-	+	+
CD 1d	-	+	-
ICAM-1 (CD54)	-	-/+	-/+
VCAM-1	-	-/+	-
LFA-3 (CD58)	-	-/+	-/+
B7-1 (CD80)	-	-/+?	-
B7-2 (CD86)	-	-/+	-/+
Apoptosis-related molecules			
Fas (CD95)	-	+	-/+
granzyme B	-	-/+	-/+
Perforin	-	-/+*	-/+
bcl-2	++	-**	++
Inflammatory cytokines			
IL-6	-	++	+
TNF- α	-/+	++	+
IL-6 receptor	-	-/+	-
TNF receptor	-/+	++	+
Others			
MUC1 apomucin	-	+	+
MUC6 apomucin	-/+	+	+
Lewis Y antigen	-	++ *	++ *
heat shock protein 27, 90	-	-/+	-/+

* Increased expression in damaged bile ducts.

** Decreased expression in damaged bile ducts.

or a set of antigenic molecules which are recognized by the immune system. All BEC's are not alike, i.e. those that line the large bile ducts are distinct from those that line the small bile ducts. Heterogeneity among BEC's may explain why only the small bile duct epithelial cells are the target of immune mediated damage in PBCs since such cells may express the target molecules not expressed by the large bile duct epithelial cells. Mechanisms involved in the disruption of the biliary epithelium in PBC, especially the association between AMA and bile duct damage remain poorly understood.

Several mechanisms have been proposed regarding the immune-mediated bile duct damage in PBC. These include: 1) T cell-mediated cytotoxicity, and 2) intracellular interaction between the IgA class of AMA and mitochondrial autoantigens in BECs during the intracellular transport, resulting in cytotoxicity. Several studies suggest that BEC are antigenically distinct since they express molecules which are associated with immune recognition of target cells such as adhesion mole-

cules, MHC antigens, and co-stimulatory molecules (Table 2). However, these molecules are not specific for PBC and are usually observed in a variety of other inflammatory hepatobiliary diseases.

Mitochondrial autoantigens; PDC-E2 and E3BP

The major autoantigens in PBC, PDC-E2 and E3BP are up-regulated in BEC when examined immunohistochemically^{31, 32, 73}. Furthermore, this up-regulation is present early in the natural history of PBC⁷⁰ and is also present in BEC in allografts of patients with recurrent PBC following liver transplantation⁷⁵, suggesting a role for these antigens in the pathogenesis and/or progression of PBC. The significance of the increased expression of PDC-E2 by BEC from PBC patients remains to be elucidated. Enhanced synthesis, impaired degradation, and/or abnormal targeting of the PDC-E2 to the surface of the BEC are possible explanations for the observations. It is not clear at present, however, whether the molecule that is detected at this special location is a whole PDC-E2, or a part of PDC-E2, or a molecule that is cross-reactive with PDC-E2. That PDC-E2 messenger RNA is undetectable in BECs argues against simple enhanced synthesis with overspill to the cytoplasmic surface²²; an alternative possibility is a trafficking defect, in which PDC-E2 is aberrantly transported to the cytoplasmic membrane. Such alteration might occur as a result of a point mutation in the mitochondrial presequence, in a manner analogous to the mistargeting of alanine¹⁰. The findings of our study using monoclonal antibodies and human combinatorial antibodies suggest that a molecule cross-reactive with PDC-E2, not PDC-E2 itself, is expressed at high levels at the luminal region of bile ducts in PBC patients⁷⁶. Analysis of AMA binding to the membrane fraction of purified BECs has suggested that AMA in patients with PBC may react with PDC-E3BP rather than PDC-E2³³.

Anion-exchanger-2 (AE-2)

AE-2 is a chloride biocarbonate exchanger present on the apical membrane of all BEC except for those lining segmental ducts⁴⁵. Expression of AE-2 is specifically reduced in PBC BEC relative to normal and other liver disease controls⁴⁸. It is not known how AE-2 could be involved in either the pathogenesis or progression of PBC but abnormal anion exchange could contribute to cholestasis and extrahepatic manifestations such as the polyglandular features frequently seen in PBC. It could also render BEC susceptible to changes in autoantigens.

Immune adhesion molecules

MHC class II (HLA-DR) and ICAM-1, which are critical for the interaction with lymphocytes are up-regulated in PBC^{1, 3, 77}, although these molecules are commonly induced in a variety of liver diseases and are not specific for PBC. The fact that MHC class-II (HLA-DR) expression is only detected during the intermediate stages of PBC and not during the early stages of the disease suggests that the expression is a secondary event⁷⁰. BECs also express high levels of several adhesion molecules, including ICAM-1 and LFA-3 that are important for mediating adhesion to lymphocytes. Work with isolated human BECs *in vitro* has demonstrated that MHC molecules and ICAM-1 are induced in these cells in response to pro-inflammatory cytokines². Taken together, up-regulation of class-II MHC antigens on BEC more likely is a consequence of the portal inflammation, not a cause of the disease.

For full-activation of lymphocytes, a co-stimulatory signal via interaction between CD28 and B7-1 (CD80) or B7-2 (CD86) is strictly required in addition to the interaction between peptide bound MHC class I and II to their cognate TCR^{43, 63}. The expression of CD80 and CD86 is largely restricted to professional antigen presenting cells such as dendritic cells. This prevents lymphocytes being activated by other cell types and reduces the chance of developing unwanted responses to self antigens. Thus, the ability of BECs to express either of the CD28 ligands CD80 and CD86 would allow them to act as antigen presenting cells and thereby to provoke and maintain a T cell-mediated response. However, there is some controversy concerning the expression of CD80/86 by BECs. Most studies suggest that they do not express CD80/86, and the most comprehensive studies by LEON and colleagues⁴⁰ have failed to demonstrate CD80 or CD86 protein or mRNA in biliary cells. However, results of one study suggests that CD80 and CD86 expression by BECs occurs early in the course of PBC.

There is now an increased understanding of how CD4⁺ T cells interact with BEC in PBC. MHC class II and ICAM-I might be sufficient for the activation of memory-T cells even when BEC lack CD28 ligands. Regarding interactions of BEC with cytotoxic T cells, the ability of BECs to act as targets for lymphocyte cytotoxicity is less controversial. BEC are susceptible to lysis by lymphokine-activated NK cells³⁹ and lymphocytes from patients with PBC are more cytotoxic for autologous BEC than controls³⁰. The TCR of this cytotoxic lymphocyte must interact closely with a putative target cell in order to recognize the cognate peptide in

association with MHC class I. This is facilitated by the expression of adhesion molecules such as ICAM-1 and LFA-3, both of which are increased on inflamed BECs in PBC.

CD1d molecules

Recently, CD1d expression on BEC of small bile ducts and epithelioid granuloma has been reported⁷¹. Especially, it is of interest that up-regulation of CD1d in bile ducts is more evident during the early stages of PBC. CD1 molecules have been shown to present non-protein microbial antigens and hydrophobic peptides in a MHC-independent manner⁵⁶. MHC-independent but CD1-restricted T cells expressing TCR $\alpha\beta$ and which are CD4⁺CD8⁻ and appear to specifically recognize non-protein ligands associated with CD1 molecules. Murine CD1-reactive T cells include a subset that bears the NK cell-associated marker. The expression of CD1d by BEC in the liver suggests that immune mechanisms through CD1 molecules could be important in the pathogenesis of PBC.

Molecules related to apoptosis: Fas antigen (CD95), perforin, granzyme B

There is evidence that BECs undergo apoptosis in PBC using in situ nick-end labeling methods for the detection of DNA fragmentation of apoptosis, although the mechanisms responsible for inducing apoptosis are presently unclear^{20, 36}. Related studies have shown increased expression of perforin and granzymes in PBC. In addition, Fas (CD95) is upregulated on the biliary epithelial cell membrane, it is possible that both of these pathways are involved^{20, 38}.

Inflammatory cytokines: IL-6, TNF- α

BECs overexpress IL-6 and TNF- α in PBC and to a lesser degree in other hepatobiliary diseases⁸⁰. TNF receptor and IL-6 receptor were also detected on these damaged bile ducts, suggesting an autocrine effect⁸⁰. The increased expression of IL-6 and TNF- α could affect the proliferation, maturation, and regulation of B cell and T cell lineage infiltrates around bile ducts²⁹. IL-6 promotes terminal differentiation of B cell and immunoglobulin secretion. TNF- α may induce the expression of adhesion molecules and HLA-DR and a variety of other antigens on the bile ducts, and may also increase the cytotoxic activities of T cells. IL-6 may be responsible for BEC proliferation via an autocrine pathway effects⁴⁷ and TNF- α may be involved in

the biliary epithelial cell damage. The autocrine role of TNF- α on cell damage, including apoptosis, had already been shown in renal epithelial cells and hepatitis B and C virus-infected hepatocytes¹⁹. TNF- α is also known to disturb the barrier function of the bile ducts, which may lead to leakage of toxic substances into the bile and a local inflammatory process and cholangitis⁴⁴.

MUC1 and MUC6 apomucins and Lewis Y antigen

Mucin, which consists of abundant carbohydrates and a core protein (apomucin), covers the mucosal surface constitutively and plays a role in protecting mucosal epithelial cells from extrinsic factors³⁵. Damaged BECs at the site of CNLDC have been shown immunohistochemically to have increased expression of MUC1 and MUC6 apomucins^{60, 61}. A similar overexpression is observed at the site of hepatitis-associated damaged bile ducts. Changes of these apomucins are not disease-specific. MUC1 apomucin is a membrane-binding type and could be a target of MHC-unrestricted cytotoxic T cell response. As MUC1 mRNA is not obviously up-regulated, immunohistochemical overexpression appears to be caused by unmasking of epitopes by deglycosylation. MUC6 apomucin is a secreted-type of a major gastric mucin and MUC6 mRNA is up-regulated as well in PBC and chronic hepatitis⁶⁰. Inflammatory cytokines may up-regulate MUC6 apomucin for protecting BECs, although the significance of their altered expression remain unknown. Lewis Y antigen, which is a carbohydrate antigen related to the Lewis blood group, is also neoexpressed in damaged BEC in PBC as well as the other hepatobiliary diseases⁵⁹. Overexpression of Lewis Y antigen is usually caused by up-regulation of α 1,3 fucosyltransferase and its expression is reportedly followed by apoptotic cell death²⁵. Therefore, Lewis Y antigen may be associated with apoptosis in damaged BEC in PBC.

Conclusion

Mitochondrial autoantigens and B cell and T cell autoepitopes have been well characterized in PBC. However, the etiology of PBC and the relation of AMA and bile duct destruction remain unclear. PBC is traditionally categorized as an autoimmune disorder. Although the proof for this contention is strong, it is also indirect and circumstantial. Direct proof, such as the ability to transfer disease by pathogenic autoantibodies or T cells is lacking. Some combination of immune

dysregulation and exposure to an etiological antigen appears to be required for full expression of disease. Establishment of an animal model of PBC would greatly facilitate future studies of the pathogenic mechanisms involved in human PBC.

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