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Cholangiocytes and primary biliary cirrhosis: prediction and predication

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Commentary

Although there have been descriptions of primary biliary cirrhosis (PBC) for more than 100 years, this condition first attracted the attention of immunologists in 1965, when patients' sera were found to have antimitochondrial antibodies (AMAs) (1). The mitochondrial autoantigens, which are highly conserved among mitochondria and even chloroplasts (2) of divergent species, have been identified as the E2 components of the 2-oxo-acid dehydrogenase family — particularly the 74-kDa E2 subunit of the pyruvate dehydrogenase complex, which catalyzes the formation of acetyl CoA. The structurally and functionally related mitochondrial proteins BCOADC-E2 and OGDC-E2, as well as the E3 binding protein (3), are also found as autoantigens in PBC patients. Work with recombinant autoantigens has shown that the presence of AMAs is virtually diagnostic of PBC and that, while they may be detected in the sera of asymptomatic people years before the clinical diagnosis of PBC, they are very rarely found in non-PBC individuals (4, 5). The B cell epitope of PDC-E2 is found on the lipoyl domains, and antibody binding occurs when the antigen is complexed with lipoic acid (6). In fact, there are only five proteins in mammals that contain lipoic acid, and four of the five are autoantigens in PBC. Lipoic acid is bound to the lysine residue of the inner lipoyl domain, where it functions as a swinging […]

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Although there have been descriptions of primary biliary cirrhosis (PBC) for more than 100 years, this condition first attracted the attention of immunologists in 1965, when patients' sera were found to have antimitochondrial antibodies (AMAs) (1). The mitochondrial autoantigens, which are highly conserved among mitochondria and even chloroplasts (2) of divergent species, have been identified as the E2 components of the 2-oxo-acid dehydrogenase family — particularly the 74-kDa E2 subunit of the pyruvate dehydrogenase complex, which catalyzes the formation of acetyl CoA. The structurally and functionally related mitochondrial proteins BCOADC-E2 and OGDC-E2, as well as the E3 binding protein (3), are also found as autoantigens in PBC patients. Work with recombinant autoantigens has shown that the presence of AMAs is virtually diagnostic of PBC and that, while they may be detected in the sera of asymptomatic people years before the clinical diagnosis of PBC, they are very rarely found in non-PBC individuals (4, 5).

The B cell epitope of PDC-E2 is found on the lipoyl domains, and antibody binding occurs when the antigen is complexed with lipoic acid (6). In fact, there are only five proteins in mammals that contain lipoic acid, and four of the five are autoantigens in PBC. Lipoic acid is bound to the lysine residue of the inner lipoyl domain, where it functions as a swinging arm to capture electrons during oxidative phosphorylation. Related work using cloned PDC-E2–specific T cell lines shows that the immunodominant T cell epitope also falls within the lipoyl domains. Interestingly, the frequency of PDC-E2–specific $CD4^+$ T cell precursors is 100-fold higher in the liver than in PBMCs (7) in PBC patients, suggesting that these cells are recruited to the liver during the progression of the disease. However, the precise pathogenic role of these T cells is still under investigation.

Despite the advances in the molecular characterization of immunoreactivity, a number of unexplained questions remain regarding the epidemiology and tissue specificity of this disease (3). Why is PBC overwhelmingly more common in women? Why is PBC not found in childhood? Why are granulomas found, and why is eosinophilia present in periductal inflammatory infiltrates? Why is PBC relatively more specific for small bile ducts than large bile ducts? Why should PBC recur following liver transplantation? Why is the incidence of PBC so much higher in families with an index case, even when there is no obvious MHC class I or class II association? What is the significance of the observation that some monoclonal antibodies to mitochondria appear to uniquely stain the bile duct cells in patients with PBC? Finally, why does the immune response induce damage to only a minor population of cells, those of the small ducts and ductules of the biliary and salivary epithelia?

Covalent modification of PDC-E2 during apoptosis

At least one of these puzzles, namely the specificity for cholangiocytes and salivary epithelium, is addressed by Odin and colleagues in this month's issue of the *JCI* (8). The authors demonstrate that, following apoptosis, PDC-E2 expression on HeLa-, Jurkat T–, and Caco-2 cell–derived membranes becomes undetectable when probed with AMAs. This loss of recognition of apoptotic cell–derived PDC-E2 by AMAs appears to reflect structural changes in the protein, rather than its actual disappearance or degradation.

Interestingly, following apoptosis in two rat cholangiocyte cell preparations (as well as in a human salivary epithelial line), PDC-E2 retains immunoreactivity with AMAs. The authors suggest that PDC-E2 retains its immunoreactivity in cholangiocytes and salivary epithelium because these cells — unlike the other cell types studied — fail to link PDC-E2 covalently to glutathione during the course of apoptosis (8). In support of this glutathiolation model, the authors show that the addition of oxidized glutathione to SDS-treated cholangiocyte cell lysates renders PDC-E2 nonantigenic when probed with AMAs. Odin et al. conclude that PDC-E2 derived from these epithelial cells remains antigenic even after apoptosis (8). The authors further demonstrate that when HeLa cells are transfected with Bcl-2, they too retain AMA reactivity to PDC-E2, mimicking what is seen in cholangiocytes. Interestingly, glutathione depletion is associated with decreased Bcl-2 expression and an increase in apoptosis in cultured cholangiocytes, and it has been proposed that glutathione functions as a cytoprotective molecule, mediating some of the antiapoptotic effects of Bcl-2 (9).

Puzzles and caveats

Because the present evidence showing the effects of glutathiolation on PDC-E2's antigenicity is convincing, it is tempting to postulate that glutathiolation of E2 proteins during apoptosis provides a protective mechanism whereby many cell types block the release of potentially pathogenic autoepitopes. However, the role of Bcl-2 in this scenario is less clear. Paradoxically, Odin et al. find an apparent lack of glutathiolation in cells that have historically demonstrated high levels of Bcl-2 (8). Perhaps analysis of

Bcl-2 expression in the cholangiocytes used in this study will prove helpful in resolving what the authors clearly state is a contradiction.

In addition, for several reasons, one must ask if the observations are generalizable to normal human bile duct cells (BECs). First, it is possible that they reflect a nonphysiological feature of the rat cholangiocytes studied. The freshly isolated cholangiocyte cell line (IBDEC) was derived following common bile duct ligation, a process known to increase the expression of Bcl-2 (10). Second, constitutive expression of Bcl-2 in cholangiocytes is consistently higher in rats than in humans, and there is little, if any, expression noted in the BECs of patients with PBC (11–14). Since glutathione levels are proportional to the level of Bcl-2, it seems unlikely that Bcl-2 expression in PBC BECs contributes to the retention of unmodified PDC-E2 (8, 9). The human salivary cell line used in the Odin study (8) is derived from a human carcinoma, and its Bcl-2 levels may far exceed those in normal salivary glands. Thus, it is possible that the "protection" of PDC-E2 through the overexpression of Bcl-2 in HeLa cells occurs through an entirely different mechanism from that in cholangiocytes. While these findings are in need of additional direct evidence, the role of Bcl-2 in this phenomenon is certainly worth further study.

Finally, several aspects of the presumed protective role of glutathiolation still need to be validated. The failure of biliary and other epithelia to carry out the covalent modification of the E2 proteins may indeed represent the first step toward autoimmune disease. However, if, as the author suggests, cholangiocytes generally have this property, what explains the relative rarity of PBC? If the initial immunizing agent is intact PDC-E2 released from apoptotic cholangiocytes, AMAs might be expected to be common in the general population. In fact, immunization with PDC-E2 in a variety of animal species and strains of mice generates a multiplicity of heteroantibodies reactive to PDC-E2, but rarely does it generate autoantibodies to self-proteins (15). Moreover, PDC-E2 has multiple reactive sites for caspases, and one would assume that, under normal conditions, PDC-E2 would be rapidly degraded during apoptosis. There are also several additional mitochondrial autoantigens in PBC, and one wonders whether the process described for PDC-E2 is applicable to them as well. Future studies must address the structural properties of BCOADC-E2, OGDC-E2, and the E3 binding protein to demonstrate whether the phenomena seen herein with PDC-E2 apply to these other mitochondrial autoantigens. Left unexplained, in any case, is why some patients develop autoantibodies to only PDC-E2, or to one or more of the other E2 molecules, rather than to the complete set of these mitochondrial proteins.

Despite these reservations, the observations by Odin et al. (8) are obviously important, as they demonstrate a unique characteristic of cholangiocytes with respect to self-antigen modification during apoptosis. Clearly, the next step toward establishing the relevance of these observations to human pathology will require the direct analysis of bile duct epithelial cells isolated from patients with PBC or other liver diseases.

1. Walker, J.G., Doniach, D., Roitt, I.M., and Sherlock, S. 1965. Serological tests in diagnosis of primary biliary cirrhosis. *Lancet.* **1**:827–829.
2. Gershwin, M.E., Coppel, R.L., and Mackay, I.R. 1988. Primary biliary cirrhosis and mitochondrial autoantigens: insights from molecular biology. *Hepatology.* **8**:147–151.
3. Gershwin, M.E., et al. 2000. Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. *Immunol. Rev.* **174**:210–225.
4. Mattalia, A., et al. 1998. Characterization of antimitochondrial antibodies in healthy adults. *Hepatology.* **27**:656–661.
5. Metcalf, J.V., et al. 1996. Natural history of early primary biliary cirrhosis. *Lancet.* **348**:1399–1402.
6. Migliaccio, C., et al. 2001. Heterogeneous response of antimitochondrial autoantibodies and bile duct apical staining monoclonal antibodies to pyruvate dehydrogenase complex E2: the molecule versus the mimic. *Hepatology.* **33**:792–801.
7. Shimoda, S., et al. 1998. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. *J. Clin. Invest.* **102**:1831–1840.
8. Odin, J.A., Huebert, R.C., Casciola-Rosen, L., LaRusso, N.F., and Rosen, A. 2001. Bcl-2–dependent oxidation of pyruvate dehydrogenase-E2, a primary biliary cirrhosis autoantigen, during apoptosis. *J. Clin. Invest.* **108**:223–232.
9. Celli, A., Que, F.G., Gores, G.J., and LaRusso, N.F. 1998. Glutathione depletion is associated with decreased Bcl-2 expression and increased apoptosis in cholangiocytes. *Am. J. Physiol.* **275**:G749–G757.
10. Stahelin, B.J., Marti, U., Zimmermann, H., and Reichen, J. 1999. The interaction of Bcl-2 and Bax regulates apoptosis in biliary epithelial cells of rats with obstructive jaundice. *Virchows Arch.* **434**:333–339.
11. Kurosawa, H., Que, F.G., Roberts, L.R., Fesmier, P.J., and Gores, G.J. 1997. Hepatocytes in the bile duct-ligated rat express Bcl-2. *Am. J. Physiol.* **272**:G1587–G1593.
12. Kuroki, T., et al. 1996. Expression of antigens related to apoptosis and cell proliferation in chronic nonsuppurative destructive cholangitis in primary biliary cirrhosis. *Virchows Arch.* **429**:119–129.
13. Iwata, M., et al. 2000. Expression of Bcl-2 familial proteins is reduced in small bile duct lesions of primary biliary cirrhosis. *Hum. Pathol.* **31**:179–184.
14. Graham, A.M., Dollinger, M.M., Howie, S.E., and Harrison, D.J. 1998. Bile duct cells in primary biliary cirrhosis are 'primed' for apoptosis. *Eur. J. Gastroenterol. Hepatol.* **10**:553–557.
15. Krams, S.M., et al. 1989. Immunization of experimental animals with dihydrolipoamide acetyltransferase, as a purified recombinant polypeptide, generates mitochondrial antibodies but not primary biliary cirrhosis. *Hepatology.* **9**:411–416.