LEADING ARTICLE

Primary biliary cirrhosis: seeking the silent partner of autoimmunity

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Primary biliary cirrhosis is a disorder characterised by an intense inflammatory response in the septal and interlobular bile ducts and is considered to be an autoimmune disease. Evidence to suggest that chronic viral infection could be a crucial element in the development of biliary epithelial cell damage and activation of the associated autoimmune response is reviewed

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antibodies reactive with the E2 subunit of pyruvate dehydrogenase complex (PDC-E2), which is considered to be the major autoantigen. However, the AMA response is polyclonal and antibodies also react with dihydrolipoamide dehydrogenase binding protein (E3BP), and the E2 subunits of branched chain 2-OADC and 2-oxoglutarate dehydrogenase complex.⁴

"A role for AMA in the pathogenesis of PBC has yet to be convincingly demonstrated"

Organ specific autoimmune diseases arise when there is breakdown in tolerance to a self antigen and loss of organ function arises as a consequence of a chronic inflammatory process that is targeted towards the self antigen. This process often occurs in the absence of a readily identifiable trigger, such as a pathogen or xenobiotic. Yet increasingly, attention is turning towards the role that pathogenic organisms may have in triggering the autoimmune response. Primary biliary cirrhosis (PBC) is a disorder characterised by an intense inflammatory response in the septal and interlobular bile ducts and is considered to be an autoimmune disease. Here, we review the evidence that suggests chronic viral infection could be a crucial element in the development of biliary epithelial cell damage and activation of the associated autoimmune response.

PBC AND THE ANTIMITOCHONDRIAL IMMUNE RESPONSE

An autoimmune component to the disease was first suggested when complement fixation tests showed that serum from patients with PBC reacted with tissue extracts,¹ and in 1965 this reactivity was found to be localised to the mitochondria using indirect immunofluorescence.² It is now apparent that antimitochondrial antibodies (AMA) are very closely linked to PBC and can be detected in more than 95% of patients with PBC. Furthermore, AMA may be detectable in peripheral blood many years before the onset of clinical, biochemical, or histological features of the disease.³ Although AMA are the autoantibodies most closely associated with PBC, any theory on the pathogenesis of the disease must take into account that other autoantibodies (such as the gp-210 reacting with nuclear pore complex) have a similar specificity for the disease.

AMA react with members of the 2-oxoacid dehydrogenase complex (2-OADC), predominantly binding to conformational epitopes of the inner lipoyl domains of the highly conserved E2 subunit. Over 95% of patients with PBC have

While AMA have functional effects inhibiting the activity of 2-OADC in vitro and a significant proportion of B cells that make up 10% of the inflammatory infiltrate present within the portal tract produce antibody reactive with PDC,⁵⁻⁶ a role for AMA in the pathogenesis of PBC has yet to be convincingly demonstrated. Furthermore, AMA cannot be detected in patients with autoimmune cholangitis, a condition that otherwise shows all the clinical, biochemical, and histological features of PBC.

In the absence of a direct role for soluble AMA in the pathogenesis of PBC, attention has turned towards the T cell response to 2-OADC. CD4+ and CD8+ T cells make up a significant proportion of the inflammatory infiltrate within the portal tracts of patients with PBC⁷ and several investigators have shown that 2-OADC reactive T cells can be cloned both from liver biopsies and peripheral blood of patients with PBC. Shimoda *et al* have used synthetic peptides and purified native protein to identify an immunodominant T cell epitope (163-176, GDLLAETETDKATT) derived from PDC-E2 and demonstrated that patients with PBC have an expanded population of CD4+-PDC-E2 163-176 specific T cells.⁸ Furthermore, PDC-E2 163-176 specific T cells were 100-150-fold more common in the hilar lymph nodes and liver than in the blood of PBC patients.⁸

Although many autoreactive T cells are deleted when they encounter self antigen in the thymus (central tolerance),⁹ many T cells potentially reactive with self antigens escape thymic deletion. Therefore, the immune system in healthy individuals contains naïve T cell populations capable of responding to a variety of autoantigens,¹⁰⁻¹² and

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Abbreviations: PBC, primary biliary cirrhosis; AMA, antimitochondrial antibodies; 2-OADC, 2-oxoacid dehydrogenase complex; PDC, pyruvate dehydrogenase complex; MS, multiple sclerosis; PLP, proteolipid protein; TMEV, Theiler's murine encephalomyelitis virus.

PDC-E2 specific T cells can be detected within the circulation of healthy controls, albeit at a lower frequency than in those individuals with PBC.⁸ However, several mechanisms of “peripheral tolerance”, including functional sequestration of self antigen or restriction of self antigen to immune privileged sites,⁹ ensure that naïve autoreactive T cells remain in an inactive state.

ACTIVATION OF THE NAÏVE T CELL RESPONSE TO AUTOANTIGENS IN PBC

Further advancement towards the understanding of the proposed autoimmune aetiology of PBC demands answers to two fundamental questions:

- (1) What is the mechanism that leads to activation of the naïve antimitochondrial immune response?
- (2) Why is PBC a tissue specific disease when mitochondrial antigens, which are the target of the autoimmune response, are expressed in all nucleated cells?

Pathological studies suggest a mechanism that explains both activation and specificity of the antimitochondrial immune response. Histological studies of liver in patients with PBC revealed that biliary epithelial cells, the target of the immune dysfunction, show aberrant cellular expression of an antigen that reacts with AMA and localises to the apical region/membrane of the cells.¹³ This phenomenon was observed early in the natural history of PBC and autoimmune cholangitis and has not been observed in other liver or autoimmune diseases. However, despite reactivity with anti-PDC antibodies, the antigen observed within the apical region of biliary epithelium does not appear to be PDC-E2, at least not in its native form. Strong evidence for this reactivity being due to either a molecular mimic or altered form of native PDC-E2 comes from the observation that only one of eight affinity purified mouse anti-PDC-E2 antibodies, derived by immunising mice with full length recombinant PDC-E2, react with this antigen, although all antibodies produced typical mitochondrial staining on liver and biliary epithelial cell sections from patients with primary sclerosing cholangitis and hepatocellular carcinoma.¹⁴ Furthermore, five anti-PDC-E2 combinatorial antibodies derived from a patient with PBC all produced identical “mitochondrial” staining patterns on Hep-2 cells but produced different staining patterns in biliary epithelial cells in liver sections derived from a patient with PBC,¹⁵ and there are differences in the electrophoretic mobility between PDC-E2 and AMA reactive antigen isolated from the plasma membrane of biliary epithelial cells derived from the liver of patients with PBC.¹⁶

It could be argued that aberrant expression of this AMA reactive material by biliary epithelium arises as a consequence of the inflammatory process. Yet intuitively it seems much more plausible that aberrant antigen expression by the biliary epithelium results in immune activation towards a previously functionally sequestered antigen. This is supported by the observation that aberrant expression of AMA reactive material may be seen in biliary epithelial cells with no evidence of an inflammatory response.⁴

A XENOBIOTIC TRIGGER?

If aberrant expression of this AMA reactive antigen is triggered by an exogenous agent, then this agent could be microbial or a xenobiotic. Both acute and chronic drug associated hepatotoxicity may be associated with autoantibodies (as is seen with, for example, tienilic acid hepatitis and antibodies to the drug metabolising enzyme cytochrome CYP2C9)¹⁷; in most cases however when the drug is withdrawn the liver damage resolves. Nevertheless, in other cases, such as the vanishing bile duct syndrome associated with amoxycillin, liver damage may progress slowly to liver failure, possibly due to enterohepatic circulation.¹⁸ In support of xenobiotics

triggering PBC, recent studies have shown that autoantibodies from patients with PBC can react strongly with mimeotopes where the inner lipoyl domain of PDC-E2 was modified by halogenated xenobiotics.¹⁹ Potential xenobiotics could be either environmental toxins or drugs. Similar mechanisms have been postulated for halothane hepatitis whereby metabolism of a drug leads to generation of a reactive metabolite which binds to the drug metabolising enzyme and so renders the enzyme a potential hapten: in the case of halothane hepatitis, evidence suggests that all those exposed to halothane can generate the antigen but in only a very small proportion of those exposed does this lead to a toxic response.²⁰

CHRONIC VIRAL INFECTION TRIGGERS THE ANTIMITOCHONDRIAL IMMUNE RESPONSE?

Despite the observation that the inflammatory response in PBC occurs in the absence of a detectable pathogen, a number of observations suggest that an infective rather than a xenobiotic agent plays a crucial role in the initiation and maintenance of the pathological process.²¹ These include clustering of cases, the increased incidence in first degree relatives without a defined strong HLA or other genetic association, lack of response to immunosuppressive therapy, observations that immigrants develop the prevalence of the disease in the country to which they have migrated, and unlike other autoimmune diseases PBC is not found in children.

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PBC also recurs after transplantation and aberrant expression of PDC-E2-like antigen is seen in biliary epithelium cells in the allografts of patients with disease recurrence. Furthermore, lymph nodes from patients with PBC (but not controls) can induce aberrant expression of PDC-E2-like antigen in biliary epithelial cells isolated from normal subjects. The observation that this effect can be abolished by irradiation, coupled to the suggestion that disease recurrence after transplantation may be greater in the face of more potent immunosuppression, implies that an infective agent is involved in the process that results in aberrant expression of PDC-E2-like antigen.

WHAT CAN BE LEARNED FROM MURINE MODELS OF ANOTHER PRESUMED HUMAN AUTOIMMUNE DISEASE?

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system that is proposed to have an autoimmune aetiology. Experimental allergic encephalitis is an animal model of MS and has been used to confirm that a number of myelin proteins are potential autoantigens in MS. Proteolipid protein (PLP) is the major component of myelin, and SJL mice immunised with PLP are particularly susceptible to developing CNS demyelination that is characteristic of MS. In SJL mice the major encephalitogenic PLP epitope is composed of amino acids 139–151 and the immunodominance of this epitope appears to be due to the expanded numbers of naïve CD4+ T cells (1/20 000) reactive to this epitope.²² Interestingly, this epitope is only expressed by the CNS specific exon of PLP. The DM20 isoform of PLP expressed in the thymus lacks residues 116–150 and as a result T cells reactive with the 139–151 epitope are not deleted within the thymus²³; manipulations that cause PLP 139–151 to be expressed within the embryonic thymus result in a significant reduction in the PLP 139–151 T cell precursor frequency.²²

Despite a significantly expanded PLP reactive T cell population, PLP is a CNS specific antigen and hence is normally sequestered from peripherally circulating T cells by the blood-brain barrier. Immunisation with exogenous PLP is one way of activating the PLP 139–151 T cells to cause CNS demyelination but PLP 139–151 T cells can also be activated following infection with Theiler's murine encephalomyelitis virus (TMEV). TMEV is a picornavirus that chronically persists in the CNS and induces a CD4+ T cell mediated demyelinating disease that resemble MS. Myelin damage is initially caused by T cells reactive with TMEV antigens but continued inflammatory demyelination results in activation of the PLP 139–151 T cell response, which can also contribute further to the demyelinating immune response.²⁴

Thus this animal model, Theiler's murine encephalomyelitis, demonstrates that persistent viral infection can lead to chronic organ specific disease associated with an autoimmune component that arises via epitope spreading to an antigen that had previously been sequestered from self reactive T cells.

PBC: PRIMARILY A VIRAL DISEASE?

We suggest that the evidence presented above is consistent with PBC being primarily due to chronic viral infection of the biliary epithelium and propose that activation of the antimitochondrial immune response occurs because viral infection leads to either expression of a molecular mimic with significant sequence homology to PDC-E2 or aberrant expression of altered native PDC-E2. Furthermore, it can be argued that the activated antimitochondrial immune response is appropriately targeted towards biliary epithelial cells that are aberrantly expressing a virally derived molecular mimic or altered form of native PDC-E2 but ignores healthy tissues that are expressing mitochondrial antigens which remain functionally sequestered from the immune system. Thus, as others have stressed, autoimmune disease is not the same as autoimmunity.²⁵

The hypothesis that PBC is primarily a viral disease appears to be controversial, not least because no pathogenic agent has been consistently implicated in the aetiology of PBC. It has been notoriously difficult to show that chronic diseases have an infectious root.²⁶ Furthermore, in addition to the presence of the antimitochondrial immune response, other evidence is cited in favour of PBC being primarily an autoimmune condition; namely an increased incidence in females, a widespread disturbance of the immune system, including the presence of elevated immunoglobulins, autoantibodies, and increased catabolism of complement, an association with other autoimmune disorders, and HLA associations.

"The hypothesis that PBC is primarily a viral disease appears to be controversial, not least because no pathogenic agent has been consistently implicated in the aetiology of PBC"

However, it should be noted that none of these observations is specific for autoimmunity. Hypergammaglobulinaemia and autoantibodies may be detected in hepatitis C infection. Increased incidences of autoimmune disorders are found in states of chronic immunodysregulation or chronic viral infection. Notably, there is an increased incidence of Sjögrens syndrome,²⁷ thyroid dysfunction,²⁸ and coeliac disease²⁹ in patients with hepatitis C (three conditions that are also notably overrepresented in patients with PBC). Finally, HLA polymorphisms also influence susceptibility to infection as well as susceptibility to autoimmunity. For example, the HLA B35 allele is associated with a worse prognosis in Caucasians with HIV infection.³⁰

Optimal treatment of any disease depends on a thorough understanding of the aetiology, and improved treatment of

PBC will require a significant improvement in our current knowledge of this seemingly enigmatic disorder. We have long recognised the association of PBC with the antimitochondrial immune response but cannot conclusively explain the processes that lead to activation of this response. Furthermore, we still need to demonstrate the effector mechanisms whereby the antimitochondrial immune response interferes with the function of biliary epithelial cells. Thus in addition to defining the precise role of the antimitochondrial immune response in PBC, it is imperative that we extend our quest more widely and explore potential roles of infective agents and xenobiotics in the pathogenesis of PBC.

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