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Review Article

Apotopes and innate immune system: Novel players in the primary biliary cirrhosis scenario

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ABSTRACT

Our understanding of primary biliary cirrhosis has been rapidly growing over the past decade and the disease is now regarded as a model for other female-predominant, organ-specific autoimmune conditions. Primary biliary cirrhosis ensues from a multi-lineage loss of tolerance to the E2 component of the pyruvate dehydrogenase complex. One of the major unanswered questions in the pathogenesis of primary biliary cirrhosis is the specificity of small intrahepatic bile ducts attack while PDC-E2 is present in mitochondria of all nucleated cells. Recent findings suggest that the uniqueness of the primary target tissue, biliary epithelium, may be of considerable importance for understanding primary biliary cirrhosis and that the biliary epithelial cell is more than an innocent victim. Rather, it attracts an immune attack by virtue of the unique apoptotic mechanisms and by the way it handles PDC-E2. Moreover, recent evidence suggests that apoptotic bodies of biliary epithelial cell are able to activate the innate immune system in the presence of anti-mitochondrial antibodies. This review article is intended to provide a critical overview of the role of apoptosis in biliary epithelial cells, the activation of the innate immune system, and its biological and clinical significance in primary biliary cirrhosis.

1. Introduction

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by progressive destruction of intrahepatic bile ducts with cholestasis, portal inflammation, and fibrosis. It may lead to cirrhosis and its complications, and eventually to liver transplantation or death. PBC is characterized by multi-lineage T and B cell responses against the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) [1–3]. There have been significant advances in our understanding of the immunobiology of PBC and, in particular, a rigorous dissection of not only the serologic abnormalities, including antimitochondrial autoantibodies (AMA), but also the definition of autoreactive CD4 and CD8 cells [4]. Further, there is increasing evidence for the interplay of genetic and environmental factors in individual host susceptibility [5]. One of the major still open questions in PBC is the selective destruction of small bile ducts in PBC despite the presence of mitochondrial antigens in virtually all nucleated cells. Of relevance, Odin et al. demonstrated that PDC-E2 remains immunologically intact in biliary epithelial cells (BECs) following apoptosis and it is still recognizable as such by AMA [6]. It is reasoned that absence of glutathiolation [6,7] may contribute to this unique feature of the BEC. Moreover, we reported that PDC-E2 is preserved in apoptotic bodies of BEC, but no other epithelial cells, during apoptosis, constituting an apotope [8], which is able to induce pro-inflammatory cytokine secretion from mature monocyte derived macrophages (MDM) from patients with PBC in the presence of AMA, including high levels of IL-12 [8–10]. We believe that the unique apoptotic features of BECs allow the exposure of a potent intracellular autoantigen to the PBC-associated multi-lineage autoimmune response that leads to the tissue-specific autoimmune injury. This scenario justifies the biliary specificity of PBC, its recurrence following orthotopic liver transplantation [11], the therapeutic failure of immunosuppressive agents [12], as well as the efficacy of ursodiol in PBC, a drug that has anti-apoptotic properties [13].

Apoptosis, the major mechanism of programmed cell death, is essential to regulate tissue growth and maintain homeostasis. The clearance of apoptotic cells is a highly regulated process, essential to avoid the outflow of intracellular content and limit the immunological response against generated antigens [14]; under physiological conditions, apoptotic cells are efficiently cleared after engulfment by 'professional' phagocytes followed by an anti-inflammatory response [15,16]. However, several reports suggest a correlation between apoptosis and autoimmunity through an

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impairment of apoptosis or an ineffective removal of apoptotic bodies leading to the release of intracellular components that are a potential source of autoantigenic stimulation [17–21] and autoimmunity onset [22–24]. The presence of intact autoantigens within apoptotic bodies [25], their participation in the processes involved in autoantigen presentation [26], and the activation of innate immunity through macrophage cytokine secretion in concert [27] are likely links between apoptosis and autoimmunity [28].

This review article is intended to provide a critical overview of current evidences on the consequences of apoptosis of BECs in PBC and the role of the innate immune system in the immune mediated destruction of the biliary tree.

2. Apoptosis in PBC

Apoptosis is essential in maintaining immune cell populations. Dying cells undergo morphological modifications including chromatin condensation, nuclear fragmentation and generation of apoptotic bodies. Furthermore, they express so called "eat-me" signals on the cell surface that allow macrophage recognition and phagocytosis [16,28]; several receptors have been reported to mediate the binding and uptake of dying cells, including phosphatidylserine [29,30]. Apoptosis is no longer considered a "trash disposal" mechanism as the clearance of apoptotic cells is a highly regulated process, essential to avoid the outflow of intracellular content and limit the immunological response against generated antigens [28,31]. One consequence of apoptotic cell ingestion is the production of immunosuppressive cytokines such as TGF-β and IL-10 [32], whereas delayed clearance leads to post-apoptotic necrosis and release of self molecules [32–34], such as uric acid heat shock proteins and HMGB-1, that promote inflammatory cytokine production [35]. In both macrophages and DCs, apoptotic cell uptake inhibits IL-12 production in response to LPS [36]. The formation of apoptotic bodies and fragments is essential during apoptosis to limit the escape of intracellular content and preclude any ensuing immunological responses against intracellular autoantigens with inflammatory reactions [34,37]. Nevertheless, apoptotic bodies and fragments can under some circumstances constitute a major source of immunogens in autoimmune diseases that involve the targeting of ubiquitous autoantigens [38,39].

Several studies have investigated apoptosis of BECs specifically in PBC. There is increased DNA fragmentation, implying increased apoptosis, in the BEC of patients with PBC when compared with normal controls [40,41]. Fas, FasL, perforin, granzyme B, and TRAIL expressed significantly greater levels on BECs of patients with PBC [41–43]. In addition, the upregulation of WAF1 and p53 related to biliary apoptosis is found in BECs of PBC [44]. TdTmediated deoxyuridine triphosphate nick-end labelling (TUNEL) staining has also shown significantly greater apoptosis of BECs in PBC than in other chronic cholestatic diseases, i.e. Primary Sclerosing Cholangitis, even when controlled for similar degrees of inflammation [41,43–45].

Although much work remains to be done in this area, recent findings suggest that intrinsic pathways induce apoptosis of BECs in PBC and that BEC apoptosis may be of considerable importance for understanding PBC. Indeed, BEC seems to be more than simply an innocent victim of an immune attack, rather, it attracts an immune attack by virtue of the unique biochemical mechanisms by which it handles PDC-E2 [46]. It is clear that mitochondrial proteins are present in all nucleated cells, yet in PBC the autoimmune attack is directed with high specificity to BECs. Notably, there are qualitative differences between the metabolic processing of PDC-E2 during apoptosis of BECs compared with other epithelial cells. The unique metabolic process of PDC-E2, during apoptosis of BECs was initially described by Odin et al. [6]. They demonstrated that PDC-E2 was found immunologically active in BECs after apoptosis, whereas in other cell types autoantibody recognition of PDC-E2, as assessed by immunofluorescence, was abrogated after apoptosis (Fig. 1). Moreover, PDC-E2 was not cleaved by caspase- or granzyme B. Loss of recognition of this antigenic epitope was apparently due to the covalent modification of a PDC-E2 sulfhydryl group by glutathione to form a mixed disulfide. In fact, both overexpression of Bcl-2 and depletion of glutathione before inducing apoptosis prevented loss of autoantibody recognition, suggesting that glutathiolation, rather than degradation or loss, of PDC-E2 was responsible for the loss of immunofluorescence signal. Recently, the same group suggested that estrogens may help preserve mitochondrial function during apoptosis [7]. Successively Allina hypothesized that the absence of lipoylysine oxidation in PDC-E2 may yield unique self-peptides following phagocytosis of the apoptotic BECs; immunostaining of samples from liver biopsies demonstrated BEC phagocytosis of apoptotic BECs in patients with PBC but not in normal livers and immunization of SJL/J mice with the reduced peptide 163–176 induced higher ratio of positive CD4+ T cell responses than those treated with the oxidated form [23].

We have recently demonstrated that PDC-E2 is not only immunologically intact during apoptosis in BECs, but it localizes in the apoptotic bodies of BECs where it is accessible to AMA recognition [8]. However, the mechanism by which PDC-E2 translocates to the cell membrane has not been elucidated. We confirmed by immunoblotting that PDC-E2 was detectable in its antigenically reactive form within apoptotic blebs from human intrahepatic BECs, but it was not detected in apoptotic blebs from the three other epithelial cell lines [8,47], whereas seven mitochondrial and four nuclear proteins were present in naive, untreated cultures of BECs and epithelial controls [47].

Moreover, recent data show that there is intense inflammatory cytokine production in the presence of the unique triad of BEC apotopes, macrophages from PBC and AMAs [9]. The cytokine secretion is inhibited by anti-CD16 and not due to differences in apotope uptake. Moreover, macrophages from PBC patients cultured with BEC apoptotic bodies in the presence of AMA markedly increased TNF-related apoptosis-inducing ligand (TRAIL) expression, which induces apoptosis in BEC [43]. Our observations may help close several remaining gaps in the understanding of PBC including the mechanisms that lead to the selective destruction of small intrahepatic bile ducts. Indeed, these findings provide a mechanism to understand the biliary specificity of PBC, the recurrence of disease following liver transplantation, the success of ursodiol in treating PBC, and emphasize a critical role of the innate immune system in the perpetuation of this autoimmune disease.

3. Immune system in PBC

The adaptive immune responses observed in PBC, including the development of autoantibodies and autoreactive CD4+ and CD8+ T cells, have been extensively studied [48]. The features of the innate response in PBC have been overlooked until recently when several studies have shed promising light on this field and ultimately suggest a determinant role of innate immunity in the onset and perpetuation of autoimmune cholangitis.

PDC-E2-specific autoreactive CD4+ and CD8+ T cells have been identified in peripheral blood from patients of PBC. Furthermore, PDCE2-specific autoreactive CD4+ T cells are 100- to 150-fold higher and CD8+ T cells are 10- to 15-fold higher in the liver compared with peripheral blood in PBC patients [49–51]. In addition to the memory CD4+ T cells, memory CD8+ T cells mediate bile duct destruction in a mouse model of PBC [47], and there is growing evidence that suggest a more direct role of cytotoxic CD8+ T cells in the biliary destruction pathway [52,53]. Compared with