EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Towards common denominators in primary biliary cirrhosis: The role of IL-12

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There have been significant advances in our understanding of the immunobiology of primary biliary cirrhosis (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 $CD8^+$ T cells [1]. Further, there is increasing evidence for the interplay of genetic and environmental factors in individual host susceptibility. One paradox has been the selected destruction of small bile ducts in PBC despite the presence of mitochondrial antigens in virtually all nucleated cells. This enigma is being addressed with the critical observation that during apoptosis, biliary epithelial cells (BECs) translocate immunologically intact PDC-E2 into apoptotic bodies, constituting an apotope, 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 interleukin-12 (IL-12) [2,3]. Importantly, data from genetic studies of humans with PBC, murine models, and *in vitro* experiments have identified the IL-12 signaling pathway as a key player in the effector mechanisms that lead to biliary destruction. In fact, genome wide association studies from three different populations have identified at least three IL-12 related genes strongly associated to PBC: *IL12A*, *IL12RB2*, and *STAT4* [4–6]. In addition, the deletion of *IL-12p40* on the transforming growth factor β receptor II dominant negative (dnTGF-βRII) murine model of PBC has established that the IL-12p40 subunit is essential for the development of autoimmune cholangitis [7].

IL-12 is a heterodimer comprised of two subunits: a 35-kDa light chain or p35 (encoded by *IL12A* gene) and a 40-kDa heavy chain known as p40 (encoded by *IL12B* gene), that are secreted by activated antigen presenting cells (APCs). IL-12 is a major cytokine for the differentiation of T helper 1 (Th1) cells and plays an essential pro-inflammatory role in both innate and adaptive immune responses [8]. Two major pathways seem to be involved in the IL-12 driven Th1 differentiation; first, the specific cellular effects of IL-12 are due mainly to its ability to induce activation of the transcription factor STAT4, which promotes interferon-γ (IFN-γ) production, and, together with the triggering of Th1 cell responses, contributes to loss of tolerance in several models of autoimmunity [9]. Of note, the p40 subunit is also a component of the dimeric cytokine IL-23, essential for the differentiation of Th17 cells. The most important target cells of IL-12 are: T cells, NK cells, and NKT cells, for which IL-12 induces proliferation, differentiation, enhancement of cytotoxicity, and the production of cytokines, particularly IFN-γ; and B cells, for which IL-12, directly or through the effects of IFN-γ, enhances the activation and production of Th1-associated classes of immunoglobulin. Further, IL-12 through STAT4 activation stabilizes t-bet, which itself drives Th1 differentiation [10]. The IL-12 heterodimer signals through the cell surface IL-12 receptor, which is composed of two chains, IL-12Rβ1 (encoded by *IL12RB1* gene) and IL-12Rβ2 (encoded by *IL12RB2* gene). IL-12R is expressed mainly by activated T cells and NK cells, but has been shown also on other cell types, such as DCs and B-cell lines.

The above observations allow one to propose a schematic pathogenesis of PBC (Fig. 1). In apoptosis of biliary epithelial cells, PDC-E2 remains intact [Fig. 1, step 1] and can provide an autoantigenic stimulus as unmodified lipoylated PDC-E2, as xenobiotic/hapten-modified PDC-E2, or as a microbial mimic of PDC-E2 [step 2]. PDC-E2 is endocytosed by an APC and leads to its maturation [step 3] and IL-12 secretion [step 4]. If the APC is activated via stimulation of TLR, self peptides will be presented in immunogenic mode via MHC class II to autoreactive $CD4^+$ T cells and via MHC class I to $CD8^+$ T cells (cross-priming) (not shown). The IL-12 heterodimer binds then its receptor on the surface of naïve T cells, NK and NKT cells which leads to Th1 differentiation [step 5], NK cytotoxicity and ultimately to IFN-γ secretion [step 6]. Cytotoxic $CD8^+$ T cells get activated [step 7] which potentiates the destruction of small apoptotic BECs expressing PDC-E2 [step 8]. INF-γ determines also macrophage activation and possibly M1 polarization [2,11]. $CD4^+$ T cells provide help to autoreactive B cells that produce AMA [step 9]. AMA can form complexes with PDC-E2 that are phagocyted by APCs via Fc receptors as another source for antigen presentation [step 10]. Thus, a multilineage anti-PDC-E2 response is generated. The BEC is vulnerable because of expression of intact PDC-E2 [see step 1], and expression of MHC molecules. Intact PDC-E2 released from damaged BEC maintains self-perpetuating disease.

Keywords: Autoimmunity; Innate immunity; Macrophages; Biliary tree; Apoptosis.
Received 25 March 2011; received in revised form 21 May 2011; accepted 28 May 2011

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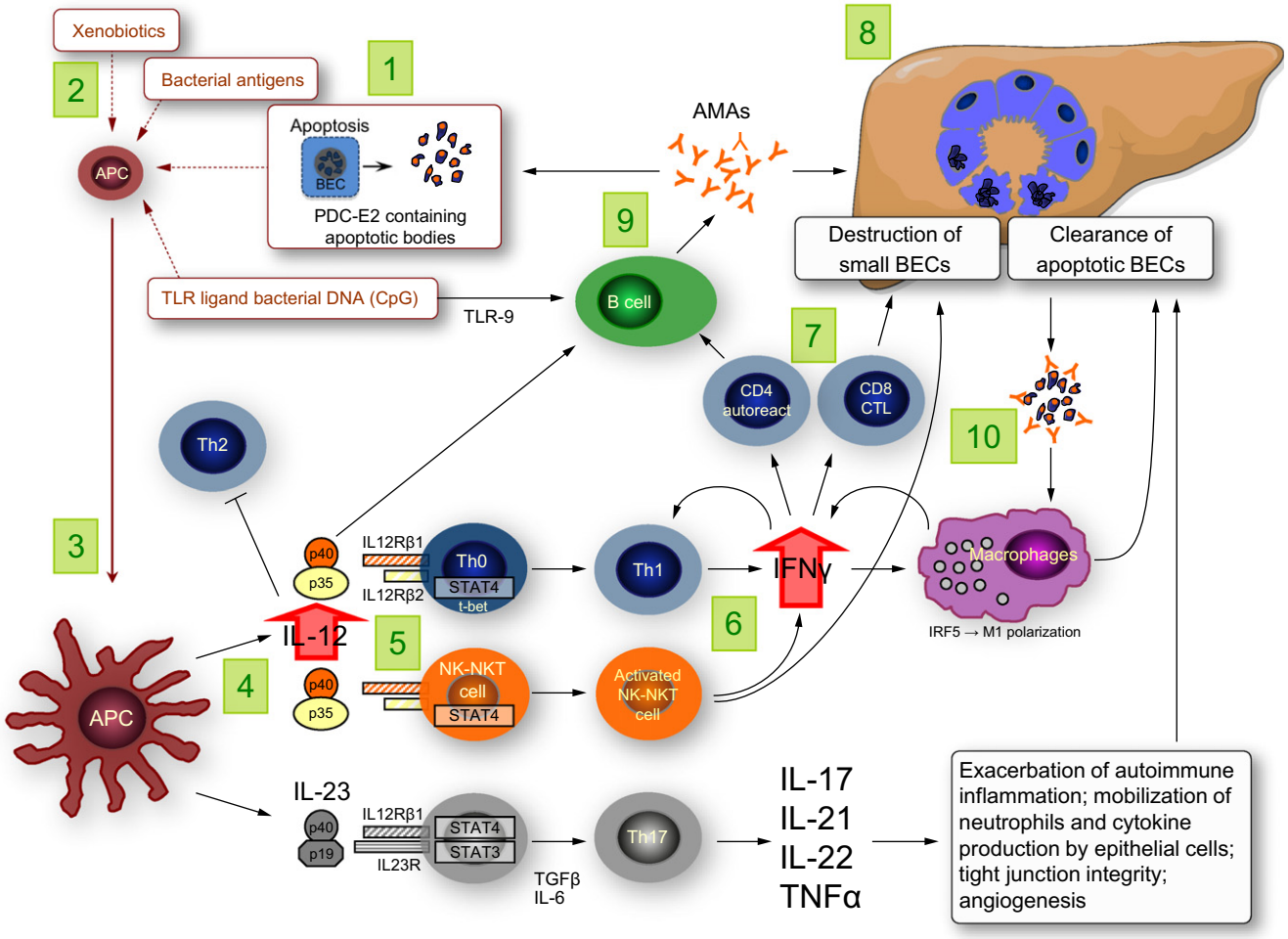

Fig. 1. Towards common denominators in primary biliary cirrhosis: the role of IL-12. BECs, biliary epithelial cells; AMA, anti-mitochondrial autoantibodies; APC, antigen presenting cell.

Interpreting the effects of IL-12 and IFN-γ in PBC is complicated, and addressing this issue will require additional studies that clarify the effector mechanisms involved in the IL-12 and the related IL-23 signaling pathways. First, based on the lack of PBC-like disease on the dnTGF-βRII animal model with deletion of *IL-12p40* [7], and the increase secretion of IL-12 from human macrophages co-cultured with apoptotic BECs [2], we have herein assumed that IL-12 production is increased in PBC; however, low levels of IL-12 could also be deleterious if there is loss of control of Th17 cells; at present, experiments correlating genes and biologic change have not been done. Second, IL-12 associations could also be consistent with an infectious etiology, however, no data linking IL-12 production to damage of a specific cell type i.e. BECs in PBC, are available. Third, other important genes e.g. *IRF5*, *CXCR5*, *NFKB1* have been shown to be genetically associated with PBC, although functional data are still missing. Importantly, high expression of IRF5 is characteristic of M1 macrophages, in which it directly activated transcription of the genes encoding IL-12 [12]. Finally, the IL-12 pathway highlights the interplay between innate and acquired immunity, and dissection of this relationship may provide opportunities for therapeutic intervention.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding of conflict of interest with respect to this manuscript.

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