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TOWARDS COMMON DENOMINATORS IN PRIMARY BILIARY CIRRHOSIS: THE ROLE OF IL-12

Ana Lleo[1,2], **M Eric Gershwin**[3], **Alberto Mantovani**[2,4], and **Pietro Invernizzi**[1,3]

[1]Center for Autoimmune Liver Diseases, Department of Medicine, IRCCS Istituto Clinico Humanitas, Rozzano, Italy

[2]Department of Translational Medicine, Università degli Studi di Milano, Rozzano, Italy

[3]Division of Rheumatology, Allergy, and Clinical Immunology, University of California at Davis, Davis, CA

[4]Department of Immunology and Inflammation, IRCCS Istituto Clinico Humanitas, Rozzano, Italy

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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 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 IL-12 (2, 3). Importantly, data from genetic studies of humans with PBC, murine models, and *in vitro* experiments have identified the interleukin-12 (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 identify 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 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 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). Moreover, IL-12 promotes interferon-γ (IFN-γ) production, which, together with the triggering of Th1 cell responses, contribute to loss of tolerance in several models of autoimmunity (9). Of note, the p40 subunit is also a

Corresponding author: Pietro Invernizzi, MD, PhD. Center for Autoimmune Liver Diseases, IRCCS Istituto Clinico Humanitas, Via Manzoni 113, 20089 Rozzano (Milan), Italy. Tel +39 02 8224 5128; Fax +39 02 8224 5191; pietro.invernizzi@humanitas.it.

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. The IL-12 heterodimer signals trough 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 specific cellular effects of IL-12 are due mainly to its ability to induce activation of the transcription factor STAT4.

The above observations allow one to propose a schematic pathogenesis of PBC. In apoptosis of biliary epithelial cells, PDC-E2 remains intact [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). 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 $CD8^+$ 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, 10). $CD4^+$ 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.

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 the IL-12 and the related IL-23 signaling pathways. Finally, IL-12 pathway highlights the interplay between innate and acquired immunity, and dissection of this relationship may provide opportunities for therapeutic intervention.

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