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Women and Primary Biliary Cirrhosis

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Abstract

Primary biliary cirrhosis occurs more frequently in women, and previous studies indicated that the average age of primary biliary cirrhosis (PBC) onset makes pregnancy in PBC patients uncommon. However, more recently, improved diagnostic testing has enabled detection of PBC in younger women, including those of childbearing age. This has led investigators to become increasingly interested in the relationship between the ontogeny of PBC and pregnancy. Published cases indicate that the typical age for pregnant women to be diagnosed with PBC is in the early 30s, and that during gestation, pruritus and jaundice are the most common symptoms. During gestation, susceptible women may experience onset of PBC resulting from the drastic changes in female hormones; this would include not only the mitochondrial damage due to accumulation of bile acids but also changes in the immune response during the different stages of pregnancy that might play an important role in the breakdown of self-tolerance. The mechanisms underlying the potential relationship between PBC and pregnancy warrant further investigation. For women first diagnosed with PBC during gestation, or those for whom first appearance of a flare up occurs during and postpartum, investigation of the immune response throughout gestation could

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provide new avenues for immunologic therapeutic intervention and the discovery of new treatment strategies for PBC.

Keywords

Autoimmunity; Female predominance; Gender and immunity; Primary biliary cirrhosis

Introduction

Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver characterized, in part, by a strong gender bias. PBC is nearly ten times more prevalent in women, and onset occurs in peri-menopausal age, usually at around 50 years [1]. However, with the recent use of serum anti-mitochondrial (AMA) autoantibodies and liver function tests—including early stage liver biopsy—the age of disease detection has expanded to include women of childbearing age [2–5]. We previously reported that more controls than patients with PBC were nulliparous [6], and there was significant association between gravidity and PBC. As with other autoimmune diseases, the disease is secondary to genetic and environmental interactions [7–9].

Gender has important implications for autoimmunity [10–15]. Pregnancy has an important impact on some autoimmune diseases, with varying outcomes (remission or exacerbation) [16–21]. The role of pregnancy in the occurrence and progression of PBC remains unclear. It has been speculated that PBC is a preexisting condition that is detected during prenatal medical supervision or that nonspecific symptoms such as pruritus are first noticed during pregnancy [1]. However, studies suggest that pregnancy does indeed create a susceptible environment for the ontogeny of PBC under certain genetic and environmental conditions. In this review, we focus on pregnancy in patients with PBC, using a systematic analysis of the literature. We discuss the possible mechanisms for pregnancy implications in PBC, including the effect of pregnancy on bile acid (BA) formation and secretion, the role of BAs in the progression of PBC, as well as sex hormones, fetal microchimerism, and the gestational immune response. We then describe how understanding the possible role of pregnancy in the etiology of PBC may help to disentangle complex host autoimmune mechanisms, provide new avenues for immunologic reconstitution in patients with PBC, and identify new targets for early PBC intervention therapy.

Pregnancy and PBC

BA Metabolism and Pregnancy

BAs, the major organic solutes in bile, are derived from two pathways. Synthesis of the first type, termed primary BAs, occurs in hepatocytes as a result of oxidation of cholesterol via the key enzyme cytochrome P450 7A1 (CYP7A1). These primary BAs replace daily BA loss via stool. The other, termed secondary BAs, is produced from the hepatocellular basolateral uptake of BAs undergoing enterohepatic circulation in the sinusoidal blood. Following synthesis, primary BAs are conjugated with taurine or glycine, then cross the canalicular membrane to be secreted in bile [22]. In the terminal ileum and colon, following

the bacterial modification, the primary BAs form secondary BAs. Ursodeoxycholic acid (UDCA), a widely used treatment for cholesterol cholelithiasis and PBC, is one of the secondary BAs and is formed as a metabolic by-product of intestinal bacteria [23].

It is widely recognized that hormones play a key role in the regulation of various metabolic processes [24]. Studies have shown that sex hormones are responsible for the differences between males and females with respect to BA metabolism [25, 26], and animal studies have provided evidence that estradiol can contribute to cholestasis [27]. During pregnancy, increased estrogen levels can result in decreased bile uptake. Hepatic estrogen conjugates inhibit bile salt uptake via the sodium-dependent bile salt transporter—Na^+/taurocholate co-transporter (NTCP)—and sodium-independent bile salt transporters—organic anion transporting proteins (OATP). These specific transport proteins are expressed at the basolateral hepatocyte membrane and contribute to the uptake of BAs [28]. Increasing estrogen can decrease the fluidity of the basolateral membrane [29–31], which causes decreased activity of the Na^+/K^+-ATPase, further hindering the sodium-dependent BA uptake into the hepatocyte.

In order to keep the balance between synthesis, uptake, and excretion of BAs, the expression of hepatobiliary transporters is tightly regulated by nuclear receptors (NRs), which provide a network of negative feedback and positive feed-forward mechanisms. During pregnancy, the nuclear BA receptor farnesoid X (FXR), the best defined NRs for BAs, regulates the transcription of the majority of genes involved in the negative feedback pathway, serving as the primary mediator of hepatic BAs [32]. The estrogen receptor alpha (ERα) participates in transporter downregulation during normal pregnancies, and activation of ERα during pregnancy is important for the repression of hepatobiliary transporters in particular. Aleksunes et al. reported that 17α-ethinylestradiol, an orally bioactive synthetic estrogen used as an oral contraceptive, was associated with downregulation of uptake gene NTCP, OATP1a1, OATP1a4, and canalicular efflux gene for the bile salt export pump (BSEP) [33]. ERα represses the function of FXR in an estradiol-dependent manner, and this inhibition may result in pro-cholestatic gene expression and heightened hepatic BA levels during pregnancy [34, 35].

Animal studies show a decline in expression of uptake and efflux transporters in the livers of pregnant mice. The reduction of transport proteins affects the uptake and excretion of BAs, BA metabolism genes, and hepatic NR genes. Aleksunes et al. demonstrated that this regulation began as early as gestational day 7 and was inversely related to increasing concentrations of circulating 17β-estradiol and progesterone as pregnancy progressed [33]. This led to reduced protection of the liver from cholestatic injury and consequent cholestatic liver disease. The authors proposed that elevated BAs during late pregnancy are probably due to increased mRNA expression of classic BA synthesis enzymes [33]. Moreover, estrogen-glucuronides in the liver are also excreted into bile by the multidrug resistance-associated protein 2 (MRP2), where they trans-inhibit the BSEP, further impacting the export of BAs [36]. These numerous factors indicate that heightened estrogen levels during pregnancy may be a factor in intrahepatic cholestasis.

Effect of BA on Biliary Epithelial Cell (BEC) Proliferation, Apoptosis, and Cytotoxicity (Fig. 1)

Impairment of bile formation or excretion processes results in accumulation of BAs in blood and hepatocytes. When the concentration of BAs exceeds the binding capacity of the binding protein located in the cytosol of the hepatocyte, hydrophobic BAs will produce toxic effects in hepatocytes [37] and BECs [38, 39]. Alpini et al. [40] provided evidence in animal studies that accumulated BAs can stimulate biliary proliferation. For example, following BA feeding, rats exhibited an increase in cholangiocyte proliferation, secretin receptor gene expression, and secretin-induced cAMP levels, similar to levels found in animals with bile duct ligation. Other studies have indicated that BAs can interact with cholangiocytes and alter cholangiocyte proliferation and bile secretion [40–42]. BAs enter cholangiocytes using the Na^+-dependent apical BA transporter (ABAT), and via this mechanism, conjugated hydrophobic BA taurocholate (TC) and taurolithocholate (TLC) could increase cholangiocyte proliferation. Accordingly, Alpini et al. found that rats fed with TC or TLC exhibited an increased number of proliferating cell nuclear antigen (PCNA)-positive cholangiocytes and bile duct proliferation compared to the control rats. Further, [^{3}H]-thymidine incorporation studies demonstrated that cholangiocytes from BA-fed rats showed increased cellular proliferation. The authors suggested that elevated BA levels could stimulate cholangiocyte proliferation and ductal secretion. To evaluate this hypothesis, they isolated small and large cholangiocytes from rats, fed the rats with TC, TLC, or BA control diet for 1 week, and determined PCNA and ABAT expression and BA transport activity. The results indicated that TC and TLC could stimulate proliferation of small and large cholangiocytes associated with protein kinase C-dependent upregulation of ABAT. Contrary to the proliferative effect of taurocholate and taurolithocholate, ursodeoxycholate and tauroursodeoxycholate inhibited the cholangiocyte proliferation [43, 44]. Inhibition of liver cell apoptosis is one of the main processes underlying the protective effect of UDCA, along with protection of cholangiocytes against cytotoxicity of hydrophobic BAs, inhibition of cholangiocyte proliferation, and stimulation of impaired biliary secretion [45]. This inhibitory effect may provide an explanation for the histological and biochemical improvement of PBC following ursodeoxycholate or tauroursodeoxycholate treatment.

In addition to stimulating biliary proliferation, BAs can also induce the apoptosis of BECs. A study by Lamireau et al. [39] indicated that although conjugated hydrophobic BA TUDC could not induce BEC apoptosis, TCDC, TDC, TLC, and TC could all induce the BEC apoptosis. While TC had only a slight effect on the induction of apoptosis, it had a striking effect on the secretion of monocyte chemotactic protein-1 (MCP-1), one of the potent chemokines that regulate migration and infiltration of monocytes [46], and interleukin (IL)-6, which is critical to triggering autoimmune reactions and contributes substantially to BEC barrier function and wound repair [47].

Specific BAs could exert potent effects on BEC; generally, taurine- or glycine-conjugated BAs seem to prevent its cytotoxic effects, and only unconjugated hydrophobic BAs (chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA)) exert cytotoxicity. Benedetti et al. reported that, in vitro, hydrophobic unconjugated BAs induced damage to intracellular organelles of BECs. Their study showed that BECs are susceptible

to the cytotoxic effects of unconjugated BAs [48] and that the primary site of damage was mitochondrial [49, 50]. Regarding the enterohepatic circulation of BAs, unconjugated BAs that have undergone de-conjugation by the intestinal bacteria are detectable in the blood circulation [51]. Benedetti et al. [48] further indicated that the mitochondrial alterations induced by DCA and LCA were similar, with a swollen appearance and electron lucent matrix. When BECs were exposed to CDCA, the mitochondria exhibited globular shapes, rarefaction of the matrix, and loss of cristae. As for the damage by BA to the apical membranes of BECs, reports indicate that only the BA lithocholate can cause changes of the BEC apical membrane [48].

The Effect of Pregnancy Hormones on Cholangiocyte Proliferation

Estrogen hormones are essential for the female reproductive system but also play an important role in the control of fundamental functions in other tissues, including the liver. Estrogens are considered immunomodulating hormones [52, 53], probably acting by potentiating the effects of growth factors and should be considered in discussing all autoimmune diseases [54]. There are three major naturally occurring estrogens: estrone (E1), estradiol (E2), and estriol (E3). E2 is the predominant estrogen during reproductive years, both in terms of absolute serum levels and estrogenic activity. During pregnancy, E3 is the predominant circulating estrogen in terms of serum levels, and during peri-menopause, E1 is the predominant circulating estrogen, though its levels drop dramatically in at menopause. Cholangiocytes are the primary targets of damage in PBC, as PBC is characterized by destruction of the BEC lining small intrahepatic bile ducts, and the progressive development of fibrotic chronic liver disease culminating in biliary cirrhosis [55]. The clinical appearance and progression of different cholangiopathies are influenced by the physiology of the female sex and changes in estrogen status in the body [56], so estrogens have been considered to play a pathogenic role in diseases, such as PBC, that preferentially affect the female sex [57].

Cholangiocyte proliferation, which acts as a repair and compensatory mechanism in the bile duct, may influence the outcome of the disease and its evolution toward the terminal ductopenic stage [58, 59]. Estrogens have been considered for many years to play a role in the development and progression of pathologies involving the biliary tree [56]. They may therefore play a role in modulating the growth of cholangiocarcinoma [60] and could modulate the disease progression in PBC [57, 61–65]. Estrogens can modulate cell growth and proliferation in target tissues expressing ERs [66–69]. It has been shown through animal studies and analysis of human liver tissue that estrogens and their receptors may influence the pathophysiology of cholangiocytes, which is characterized by cholangiocyte injury and proliferation [70–73]. Once bound by estrogen, the ER undergoes conformational changes that allow the receptor to bind DNA elements in target gene promoters and activate transcription [66–69]. In this way, estrogens work through interaction with the ERs to induce transcriptional regulation. Most cells of the human immune system express ERs, including $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, natural killer (NK) cells, dendritic cells (DCs), and macrophages. This suggests that estrogens may be able to modulate the function of these immune cells [74, 75]. ERs have two different forms, ERα and ERβ, that may play different roles in gene regulation. Paech et al. [76] found that, when complexed with

the natural hormone estradiol, the two forms produced opposite effects. When bound to ERα, 17β-estradiol activated transcription, whereas interaction with ERβ led 17β-estradiol to inhibit transcription.

Cholangiocytes are the primary cells targeted by immunopathology in PBC. During PBC progression, cholangiocytes will undergo proliferation as well as loss. Alvaro et al. [70] reported that ERs are not found in cholangiocytes of normal liver but are significantly positive in cholangiocytes from patients with PBC. They demonstrated that in PBC, the presence of ERα increased from stage I to stage III, but in stage IV, ERα was absent in association with maximal ductopenia. ERα positivity in cholangiocytes of patients with PBC was markedly lower than primary sclerosing cholangitis and alcoholic cirrhosis. The low expression of ERα in PBC and its disappearance in the advanced histological stages suggests that an estrogenic deficiency could favor the evolution of this disease toward ductopenia. These reports indicate that estrogens, in conjunction with various growth factors, may play an important role in sustaining cholangiocyte proliferation and in depressing cholangiocyte apoptosis [77–79]. However, some results provide evidence contrary to this hypothesis. Floreani [57] reported that dehydroepiandrosterone sulfate, a metabolite of dehydroepiandrosterone/prasterone (DHEA-S), serum levels were significantly higher in PBC subjects and were higher in precirrhotic than in cirrhotic patients. Alvaro et al. [70] found the serum levels of 17β-estradiol in PBC stage IV were significantly higher than PBC stage I. Alvaro et al. suggested that this discrepancy may be a consequence of cholestasis and liver failure. Despite these observations, it seems that endogenous estrogens could contribute to the PBC progression. The above studies measured serum levels of estrogen rather than the presence of the ERs. Granulomas are also a feature of PBC but the mechanisms involved are still unclear [80]. Clearly, more work is needed to resolve these findings.

Parikh-Patel [6] showed a higher frequency of past oral contraceptive use in patients with PBC than controls, though the difference was not significant. Previous studies from our group [81] have indicated that the history of oral contraceptives may not affect the onset of PBC but have shown significant differences between PBC and controls using hormone replacement therapy, regardless of past or current use of oral contraceptives. However, Corpechot et al. [82] considered the use of oral contraceptives to be a putative protective factor instead of a risk factor. To date, no studies have been able to clearly define the link between the use of oral contraceptives or hormone replacement therapy and PBC.

Progesterone, a steroid hormone synthesized by the corpus luteum of the ovaries and adrenal glands during pregnancy, as well as the central and peripheral nervous system may also play a role in cholangiocyte proliferation [83, 84]. A study by Glaser et al. [85] reported a significant downregulation of progesterone secretion in cholangiocytes isolated from rats with experimental bile duct ligation (BDL). Rat cholangiocytes express both nuclear and membrane progesterone receptors, and while chronic administration of progesterone to normal rats appears to stimulate biliary proliferation, this proliferation can be partially prevented by the administration of a neutralizing anti-progesterone antibody. Cholangiocytes from both female and male rats possess the enzymatic pathway

for progesterone steroidogenesis and secretion, suggesting that progesterone may be an important regulator of cholangiocyte proliferation.

Prolactin is another hormone responsible for regulating the growth of female cholangiocytes. A study by Taffetani et al. [86] indicated that prolactin stimulates normal cholangiocyte growth by an autocrine mechanism. Normal female rat cholangiocytes express prolactin receptors, whose expression increased following BDL. They reported that long receptor isoform is predominant in cholangiocytes but not in hepatocytes, indicating that the actions of prolactin on the liver are cell type-specific. The administration of prolactin to normal female rats increased cholangiocyte proliferation, while administration of an anti-prolactin antibody to BDL female rats led to decreased cholangiocyte proliferation [86]. Thus, altered prolactin production may affect disease progression, and regulation of prolactin secretion may be helpful for the management of cholangiopathies affecting female patients.

Cholangiocyte proliferation is a stepwise process involving liver fibrosis during acute and chronic cholestasis and finally leading to periportal fibrosis and eventually biliary cirrhosis [87]. Steroid hormone stimulation of cholangiocyte proliferation might contribute to the crosstalk occurring during cholestasis [85, 88]. In summary, these combined studies suggest that proliferating cholangiocytes may have a role in the induction of fibrosis, either directly or indirectly ways [89–92].

Clinical Evidence of Pregnancy-Associated PBC

Cases of Pregnancy with PBC (Table ;1)—Earlier studies about PBC have been limited to case reports and small cohort studies. It was in 1950 that Ahrens et al. first coined the term PBC [93]. After a detailed study of fertility and PBC, the authors found that PBC was associated with infertility—most of the patients had scanty, irregular, or profuse periods and even progressed to amenorrhoea—but produced no irregularities in patients who did become pregnant. Consistent with these early results, Olsson [94] reported four healthy pregnancies in three patients with PBC; all the pregnancies occurred after diagnosis, the reproductive ages were 22, 25, 33, and 36, and all pregnancies ended with normal deliveries. In contrast, Whelton [95] reported five patients with PBC, four of whom went to term and had vaginal deliveries, and all of whom presented jaundice in the last trimester. The authors suggested that the endogenous sex hormones associated with pregnancy may have had an exacerbating effect on the preexisting cholestatic liver disease. In 1989, Nir [96] reported a 20-year-old pregnant woman diagnosed with PBC who experienced different clinical symptoms, including not only jaundice but also itching. In 1995, Rabinovitz [97] reported a more severe case in which a PBC patient, diagnosed with PBC during the third trimester of pregnancy, gave birth to a normal healthy child before her disease rapidly deteriorated and she had to be listed for liver transplantation. These reports indicate that there may be a relationship between PBC and pregnancy.

Despite reports from these and other studies, we have few clues as to the relationship between pregnancy and PBC. It should be noted that the concordance of PBC in twins is about 60 % [111].

In 2002, standardized questions were used to compare 182 cases of PBC with 225 age- and sex-matched friend controls to examine the role of reproductive factors in PBC. The results showed a significant association between pregnancy and PBC, where patients with PBC had more pregnancies than controls, and had their first child at an earlier age [6]. In 2013, Trivedi et al. [2] performed a retrospective analysis of women with PBC during pregnancy. They identified 50 pregnancies in 32 women and found that liver biochemistry remained stable in 70 % of patients throughout pregnancy, and 72 % had a flare in biochemical disease activity postpartum. Moreover, only 6 % of patients developed progressive disease after delivery. In 2014, Efe published another retrospective study [5], which indicated that 30 % of the pregnancies with PBC were associated with biochemical flares but no maternal deaths. Seventy percent of patients exhibited clinical improvement or stabilization, and 60 % of pregnant patients showed postpartum flare. One patient was referred for liver transplantation after delivery. These studies suggest that female hormones during pregnancy likely drive the immunological changes behind, and contribute to, PBC flare and progression.

In pregnant women, intrahepatic cholestasis of pregnancy (ICP), a pregnancy-related liver disease presenting similar clinical features and onset time during gestation to PBC [112], is the most frequent cause of cholestasis and may therefore warrant further investigation [113]. Like PBC, ICP is characterized by pruritus, elevated alkaline phosphatase (AP), and an increase in the levels of BAs. At this point, UDCA is the most effective therapy. However, whether there is a correlation between ICP and PBC has not been established. Ropponen et al. [114] analyzed the intrahepatic cholestasis of pregnancy as an indicator of liver biliary diseases; when analyzed separately for PBC alone, the rate ratio did not reach statistical significance, but need to rule out the possibility of the small number of occurrences [115]. Otherwise, during normal pregnancy, it has been demonstrated that serum alkaline phosphatase levels can increase significantly in the third trimester [116]. Serum alkaline phosphatase elevation is one of the most important biochemical indicators of cholestasis and PBC [117], but during pregnancy, this elevated serum alkaline phosphatase may be due to production by the placenta [118]. Pregnancy may contribute to the onset of cholestasis in susceptible individuals under certain genetic and environmental conditions, leading to the detection of hepatic diseases during pregnancy.

Case Studies of PBC in Different Female Life Cycles

Based on the observations above, we hope to elucidate whether the changes in female hormones during pregnancy can drive the susceptible individual’s onset and progression of PBC. We therefore consider other phases of the human life cycle that may help to elucidate the effect of female hormones on PBC.

PBC has seldom been reported in childhood, but during pubertal development, peri-menstrual period, pregnancy, and peri-menopausal periods, when females experience hormonal changes, the risk of developing PBC notably increases. Dahlan et al. [119] were the first to report two pediatric-onset AMA-positive patients with PBC: both were overweight girls, diagnosed with PBC at 15 and 16 years of age, respectively. One was reported stable after starting treatment with UDCA, but the other needed liver

transplantation. What could explain the early onset and rapid progression of the disease in these patients? The age of puberty appears to be related more to body weight than to chronologic age; heavier minimum weight for height is necessary for the onset and maintenance of regular menstrual cycles in girls over 16 years of age [120], so the patients were experiencing hormonal changes and were consequently at greater risk for developing progressive PBC. Increased awareness of early-onset PBC may create opportunities for preventative measures, including intervention for individuals exhibiting certain risk factors. Following Dahlan's report, Floreani et al. [121] described a diagnosis of PBC in a 17-year-old girl, the third youngest case of PBC ever reported. This patient appeared healthy but presented with premenstrual itching 1 year prior to diagnosis. Female hormones undergo dramatic fluctuations during menstrual cycle; in order to prepare for pregnancy, estrogen and progesterone hormones are released in the premenstrual phase of the cycle to stimulate the lining of the uterus. If a pregnancy does not occur, the hormone levels decline. The onset of PBC in this patient occurred during a time period that was characterized by rapid female hormonal changes, suggesting that the sex hormone changes accompanying the premenstrual period may have contributed to her PBC onset. Shah et al. [122] reported a case in which a 36-year-old pregnant patient with PBC who stayed off of UDCA after pregnancy exhibited normal liver function during pregnancy but sharp deterioration postpartum. This sharp deterioration may have been the result of the hormonal changes occurring postpartum. And as we know, PBC onset usually occurs in peri-menopausal age at around 50 years [1], and based on the data discussed, sex hormone changes in different female period of women may contribute to an increased risk of PBC onset or progression in susceptible individuals.

Fetal Microchimerism and PBC

The incidence of autoimmune disease varies relative to the onset of reproductive function. Multiparous women may experience significantly altered susceptibility to PBC, as prevalence of PBC has been associated with greater number of pregnancies [6], while nulliparous women have been negatively associated with PBC [81]. These associations suggest that PBC may be a pregnancy-related liver disease, but how pregnancies affect PBC has yet to be determined. Pregnancy has both short-term effects and long-term consequences on the female body; in addition to the physiological changes that accompany pregnancy, fetal cells traffic into the maternal circulation, where they can persist in blood and tissues for decades [123]. The presence of small numbers of allogeneic fetal cells in the maternal system is termed fetal microchimerism, and these fetal invading cells may be the cause of the pregnancy-associated differences observed in patients with autoimmune diseases. It would be advantageous to the study of autoimmune diseases if we could elucidate how the woman's immune system responds to these "foreign" cells. In 1996, Nelson et al. proposed that fetal microchimerism might, in part, explain the female predilection to autoimmune disease that women retain following pregnancy [124]. In a follow-on study in 1998, it was discovered elevated levels of fetal microchimerism in the blood of women with scleroderma compared to healthy women [125]. This was the first study to explore the radical concept that many autoimmune diseases may be caused by microchimerism, and subsequent studies have extended this line of inquiry to investigate the potential role of microchimerism in PBC. Kobayashi [126] reported that maternal microchimerism is present within the livers of patients with progressive postnatal type biliary atresia, suggesting that biliary atresia

could be a graft-vs-host disease (GVHD) triggered by maternal microchimerism that is masquerading as an autoimmune reaction.

PBC shares important features of autoimmunity with chronic GVHD [127, 128]. Clinical onset in both diseases is characterized by a significant rise in alkaline phosphatase, bilirubin, hypergammaglobulinemia, and autoantibodies including anti-mitochondrial antibodies, with only mild elevation in transaminases. Both are associated with dry gland syndrome and other co-occurring autoimmune diseases. The histologic characteristics include mononuclear cell infiltrates into the portal triad with bile duct injury, bile duct loss, and proliferation of bile ductules. Primary biliary cirrhosis is associated with human leukocyte antigen (HLA) class II genotypes, including HLA-DR molecules. Nelson [124] has suggested that fetal microchimerism might be involved in the etiopathogenesis of some autoimmune diseases, including PBC, probably through initiation of a GVHD-like response [129]. This work is of particular interest for PBC because fetal cells may contribute to alteration of the host response to normal environmental pathogens, during pregnancy and in the following years. Such fetal cells could induce loss of tolerance to fetal antigens that allows the maternal immune system to react to microchimeric fetal cells, and thus, fetal hepatocytes may act as immune targets or as a source of foreign antigen leading to chronic inflammatory diseases like PBC. However, Invernizzi et al. found that in addition to fetal cells, fragments of fetal DNA are also present in maternal circulation. These data do not support the hypothesis that fetal microchimerism plays a significant role in the onset or progression of PBC. The peripheral blood of the PBC women did not contain a higher frequency of male DNA than that of the healthy controls, and the presence of male DNA in patients with PBC was not associated with any particular characteristics of the disease [130]. Furthermore, though some studies reported fetal cells and DNA in liver samples from patients with PBC, most also found fetal microchimerism in control livers as well, offering no clear indication that fetal cells present in the liver contribute to the disease [131–135]. Thus, previous studies suggest that analysis of the biological effects of fetal microchimerism in maternal liver requires further study. More recently, workers have emphasized the role of the X-chromosome and also epigenetic alterations [136, 137].

Immune Response in Pregnancy and PBC (Fig. 2)

Unlike organ transplants, foreign genes donated by the father during pregnancy can be tolerated by the female immune system. Consequently, the innate immune system might play an important role in pregnancy progression. NK cells recognize the major histocompatibility complex (MHC) class I chain-related gene B (MICB), which is a membrane-bound glycoprotein involved in both innate and adaptive immunity through its interaction with natural killer group 2D (NKG2D) receptor. However, during pregnancy, MHC class I expression is reduced on the syncytiotrophoblast and instead express HLA-G. This inhibits NK cells from binding to activating their killer cell immunoglobulin-like receptors (KIR) [138], thereby blocking the cytolytic activity of NK cells and promoting immune regulation [139]. In addition to the low MHC expression, Thellin and Heinen found that during pregnancy, the transporters that have been previously associated with PBC-specific antigen processing were poorly functioning [140]. Interestingly, E2 was found to suppress MICB mRNA as well as surface protein levels in a dose-dependent

manner. Estrogens also appear to have an influence on NK cell number [141]. Studies have reported that the number of peripheral NK cells decreases with an increase in estrogen concentration during pregnancy [142–144]. Studies have additionally reported heightened levels of T helper 2 (Th2) cytokines and with a decline in T helper 1 (Th1) cytokines during pregnancy [145, 146], which further decreases NK cell numbers and NK cytotoxic activity thereby reducing the opportunities for loss of immune tolerance. This inhibition of NK cytotoxicity will abate approximately 6 months postpartum [147]. The breakdown of immune tolerance to self-PDC-E2 and the adaptive multilineage anti-mitochondrial response are the key steps toward PBC onset and usually appear as an extended clinical prodrome. The mechanism for loss of self-tolerance in PBC is as yet unclear, and direct cytotoxic activity against autologous BEC has yet to be demonstrated. Thus, the arm of the immune system responsible for bile duct destruction remains to be determined.

Toward that goal, NK, natural killer T cell (NKT), and monocytes are all important cells involved in innate immune response, and each of them may be involved in the pathogenesis of PBC. The role of NK cells in the immunopathogenesis of PBC is not yet clear, though our preliminary data suggest that the number and mean frequency of NK cells in blood of patients with PBC is greater than control subjects. Additionally, isolated NK cells from patients with PBC had significantly higher natural cytotoxic ability and higher expression of perforin, and NK cells were recruited to liver more efficiently, potentially leading to higher cytotoxicity and exacerbated hepatic damage [148]. After 9 months of pregnancy, the natural cytotoxic ability of NK cells returns to normal levels [147], but whether this is associated with postpartum flare up has not been determined.

NKT refers to CD1d-restricted T cells, which are of particular interest due to the recent identification of a specific subset of invariant natural killer T cells (iNKT) that link the innate and adaptive immune responses [149]. iNKT cells exert important regulatory functions through their capacity to produce both Th1 and Th2 cytokines. PBC is characterized by Th1-polarized T cell responses [150], implicating a role for NKT cells in the regulation of autoimmune diseases. Activation of iNKT cells is a critical factor in modulating the natural history and acceleration of the disease, as the ligand-activated CD1d-restricted NKT cells are of great importance to PBC initiation and the evolution from subclinical to clinical disease [151–153]. Our group found that transformation of growth factor beta receptor II in a dominant-negative mouse model enabled NKT to attenuate the development of PBC. We have demonstrated that NKT cells can exacerbate liver injury in PBC [153], and that in patients with PBC, the NKT cell number and hepatic CD1 expression were elevated [151, 152]. The proportion of NKT cells was significantly decreased in the liver of patients with early PBC, but increased with advanced PBC, and the proportion of activated Fas ligand (FasL)-positive NKT cells was significantly increased in the livers of patients with advanced compared to early PBC. Activated NKT cells may therefore contribute to the biliary epithelial cell death resulting in the progression of PBC [154]. E2 can increase the number of NKT cells producing interferon (IFN)-γ and the IFN-γ mRNA expression level [155]. Interestingly, in pregnancy, the numbers of iNKT cells in the peripheral blood do not change, and they become less able to produce the type 1 cytokine IFN gamma between the first, second, and third trimester; however, the cells become very activated in the third trimester [156].

Monocytes are another class of key innate immune effector cells that produce cytokines and chemokines upon activation. E2 has a direct role in the modulation of monocyte immune function [157]. Luppi et al. [158] reported that in normal pregnancy, the number of monocytes was stable throughout gestation, but monocytes could produce higher IL-12 and IL-1β, especially in late pregnancy. Comparatively, monocytes from patients with PBC seem to produce higher relative levels of pro-inflammatory cytokines, especially IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, which are critical to the inflammatory response that may be essential in the breakdown of self-tolerance [159]. However, whether monocytes participate in the flare observed in late PBC pregnancy is still unclear.

Women also tend to have higher levels of immunoglobulins. During pregnancy, asymmetrical IgG molecules increase in serum, and the placenta is capable of releasing factors that can regulate the relative proportion of asymmetrical IgG molecules and induce modifications of the produced antibodies. Asymmetrical IgG molecules are univalent antibodies and therefore act as antigen blockers [160]. In early pregnancy, IgM levels decrease immediately but then rise rapidly following delivery [161]. As we know, in patients with PBC, beyond cholestasis and the presence of mitochondrial antibodies, the common feature is high levels of IgM. Little is known about the role of hyper-IgM in the pathogenesis of PBC, but the elevated levels of serum IgM in patients with PBC seem to be related to high expression of CD40L that plays an important role in modulation of BEC apoptosis in PBC [162].

$CD4^+CD25^{high}$ regulatory T cells (Tregs) are a subset of T cells that can regulate the cellular immune response [163]. Some studies have shown an increase in the peripheral $CD4^+CD25^+$ Treg cell pool during pregnancy [164–166], and Tillburgs et al. [167] indicated that the percentages of $CD4^+CD25^{high}$ T cells in decidua were significantly higher than that in peripheral blood. Mjosberg and colleagues [168] reported that during pregnancy, Tregs contribute to strict regulation of both Th1-like and Th2-like anti-fetal immune reactions, but Th2-like cells can escape the suppression of Tregs. This would allow for increased IL-4 and potential regulation of potentially detrimental IFN-γ production. However, they did not find any evidence for altered Treg numbers or function during pregnancy. Impairment of $CD4^+CD25^{high}$ Tregs could also play an important role in the breakdown of self-tolerance [163, 169]. Forgers et al. [170] reported that numbers of $CD4^+CD25^{high}$ Tregs were inversely correlated with disease activity of rheumatoid arthritis in the third trimester and postpartum, but how the numbers of $CD4^+CD25^{high}$ Treg cells change in the flare up in the third trimester of pregnancy with PBC warrants further study.

Estrogen modulates the cell-mediated immune response by inducing TH2 cytokines to inhibit TH1 responses [171]. Successful implantation depends upon these cytokines to create an optimal environment [172, 173]. Pregnant females are biased toward Th2-type immunity rather than Th1-type immunity; during pregnancy, IL-10, IL-4, etc. (Th2-associated cytokines) increased, while T helper cells (Th1-associated cytokines), like IFN-γ, IL-1, etc., were decreased. The authors also observed reduced numbers of pro-inflammatory cytokines like TNF-γ and IL-12 [145, 146]. In addition to estrogen, progesterone serves as an important effector in establishing Th2 bias in pregnancy. It exerts immunomodulator action by inducing blocking factors expressed on lymphocytes, altering cytokine secretion,

and reducing the production of pro-inflammatory cytokines [174]. Progesterone was found to specifically block trophoblast elicit Th1 immunity and inhibit IL-10 production but upregulate tumor growth factor (TGF)-β secretion [175].

Prolactin has been shown to have a role in immunomodulation [176]; during late pregnancy, prolactin will reach its highest levels to enable female mammals produce milk [177]. Matalka [178] reported that in addition to its role in milk production, prolactin can enhance the function of Th1-mediated response by increasing production of IFN-γ, IL-12p70, IL-10, [179] but not TNF-α, in whole blood [178].

T cell-mediated immune response has been considered to play an important role in the pathogenesis of PBC [180, 181]. The destruction of the biliary tract in PBC is thought to be mediated by auto-reactive liver infiltrating $CD4^+$ T helper cells [182]. In the majority of patients with PBC, $CD4^+$ and $CD8^+$T-cells reactive with PDC are present in the peripheral immune system and liver [183], but cytotoxic T lymphocytes (CTL) are thought to be directly involved in the tissue injury in patients with PBC [184]. Kita reported a 10-fold increase in the frequency of auto-reactive CTL in the liver as compared to the blood in patients with PBC [185]. Nagano [186] reported that IFN-γ and IL-5, IL-6, IL-10, IL-12, and IL-15 were all expressed in most liver biopsies of patients with PBC where IFN-γ was highly expressed in contrast to the regulatory cytokine, IL-10, and IL-2 and IL-4 were rarely detected. The authors believed that both Th1 and Th2 cytokines might play a role in the pathogenesis of PBC. For this study, patients with PBC were all selected at an early stage, so these results require further study in later stage samples. Soon after, Itoh [187] and colleagues used a murine model of PBC to clarify the relationship between the by $CD4^+$ T cell cytokine profile and the formation of hepatic lesions. They investigated the elevation of IFN-γ mRNA expression at an early phase before the appearance of nonsuppurative destructive cholangitis. IL-10, which can stimulate antibody production and inhibit the function of Th1 cells and developing fibrosis, showed delayed expression.

Th17 cells can selectively produce IL-17, and play a critical role in the induction of inflammation and the pathogenesis of autoimmune diseases [188, 189]. Th17 cells and Tregs share a requirement for TGF-β, high TGF-β concentrations induce Tregs, and terminal differentiation of Th17 cells requires IL-1β and TGF-β [190]. Studies indicate that during normal pregnancy, the population of peripheral blood Tregs cells increases, while the number of circulating Th17 cells decreases [191]. This ration would be reversed in the case of embryonic death, where the Th17 cells are increased and Tregs decreased [192]. In patients with PBC, the circulating Th17 cells were increased and Tregs were decreased [193], and recently, our group found that this Th1/Th17 imbalance was relevant to the pathogenesis of PBC. Whether a flare up during or postpartum in patients with PBC is associated with changes in the ratio of Th1/Th17/Tregs needs further study.

Future Directions

One of the major difficulties in PBC is the latency time between the onset of the loss of tolerance and the clinical presentation. There is increasing evidence, with respect to autoantibodies, that this loss of tolerance occurs for many years before the onset

of symptoms. This makes identification of etiological factors more difficult. There are, however, mouse models of PBC and these should be explored in more detail [194–198]. In addition, there is increasing evidence for the role of specific lymphoid subpopulations as effector mechanisms in PBC, but the role of hormones in the modulation of these pathways remains enigmatic [199, 200]. We also note that researchers should take advantage of newer technologies in both genomics and proteinomics to identify effector pathways that may also lead to therapeutic tools [201]. PBC is in the crossroads now in which there is considerable data on basic science but a relative lack of translation to therapies that will benefit patients. We submit that it is time to apply these advances in basic science to patients and begin further efforts in such translation.

References

1. Kaplan MM, Gershwin ME (2005) Primary biliary cirrhosis. N Engl J Med 353:1261–1273 [PubMed: 16177252]
2. Trivedi PJ, Kumagi T, Al-Harthy N, Coltescu C, Ward S, Cheung A et al. (2014) Good maternal and fetal outcomes for pregnant women with primary biliary cirrhosis. Clin gastroenterol and hepatol: the off clin practice j of the Am Gastroenterol Assoc 12:1179–1185
3. Prince MI, James OF (2003) The epidemiology of primary biliary cirrhosis. Clin in Liver Dis 7:795–819
4. Floreani A, Infantolino C, Franceschet I, Tene IM, Cazzagon N, Buja A et al. (2014) Pregnancy and primary biliary cirrhosis: a case–control study. Clinical reviews in allergy & immunology
5. Efe C, Kahramanoglu-Aksoy E, Yilmaz B, Ozseker B, Takci S, Roach EC et al. (2014) pregnancy in primary biliary cirrhosis. Autoimmunity reviews
6. Parikh-Patel A, Gold E, Utts J, Gershwin ME (2002) The association between gravidity and primary biliary cirrhosis. Ann Epidemiol 12:264–272 [PubMed: 11988415]
7. Miller FW, Pollard KM, Parks CG, Germolec DR, Leung PS, Selmi C et al. (2012) Criteria for environmentally associated autoimmune diseases. J Autoimmun 39:253–258 [PubMed: 22771005]
8. Selmi C, Leung PS, Sherr DH, Diaz M, Nyland JF, Monestier M et al. (2012) Mechanisms of environmental influence on human autoimmunity: a National Institute of Environmental Health Sciences expert panel workshop. J Autoimmun 39:272–284 [PubMed: 22749494]
9. Miller FW, Alfredsson L, Costenbader KH, Kamen DL, Nelson LM, Norris JM et al. (2012) Epidemiology of environmental exposures and human autoimmune diseases: findings from a National Institute of Environmental Health Sciences Expert Panel Workshop. J Autoimmun 39:259–271 [PubMed: 22739348]
10. Quintero OL, Amador-Patarroyo MJ, Montoya-Ortiz G, Rojas-Villarraga A, Anaya JM (2012) Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. J Autoimmun 38:J109–J119 [PubMed: 22079680]
11. Menon R, Di Dario M, Cordiglieri C, Musio S, La Mantia L, Milanese C et al. (2012) Gender-based blood transcriptomes and interactomes in multiple sclerosis: involvement of SP1 dependent gene transcription. J Autoimmun 38:J144–J155 [PubMed: 22119415]
12. Pollard KM (2012) Gender differences in autoimmunity associated with exposure to environmental factors. J Autoimmun 38:J177–J186 [PubMed: 22137891]
13. Pennell LM, Galligan CL, Fish EN (2012) Sex affects immunity. J Autoimmun 38:J282–J291 [PubMed: 22225601]
14. Shoenfeld Y, Tincani A, Gershwin ME (2012) Sex gender and autoimmunity. J Autoimmun 38:J71–J73 [PubMed: 22222237]
15. Amur S, Parekh A, Mummaneni P (2012) Sex differences and genomics in autoimmune diseases. J Autoimmun 38:J254–J265 [PubMed: 22204900]
16. Ostensen M (1992) The effect of pregnancy on ankylosing spondylitis, psoriatic arthritis, and juvenile rheumatoid arthritis. Am j of reprod immunol: AJRI 28:235–237 [PubMed: 1285888]

17. Kaaja RJ, Greer IA (2005) Manifestations of chronic disease during pregnancy. JAMA: the j of the Am Med Assoc 294:2751–2757
18. Ostensen M, Villiger PM, Forger F (2012) Interaction of pregnancy and autoimmune rheumatic disease. Autoimmun Rev 11:A437–A446 [PubMed: 22154710]
19. De Carolis S, Salvi S, Botta A, Garofalo S, Garufi C, Ferrazzani S et al. (2014) The impact of primary Sjogren's syndrome on pregnancy outcome: our series and review of the literature. Autoimmun Rev 13:103–107 [PubMed: 24044939]
20. Gatto M, Iaccarino L, Canova M, Zen M, Nalotto L, Ramonda R et al. (2012) Pregnancy and vasculitis: a systematic review of the literature. Autoimmun Rev 11:A447–A459 [PubMed: 22155197]
21. Westbrook RH, Yeoman AD, Kriese S, Heneghan MA (2012) Outcomes of pregnancy in women with autoimmune hepatitis. J Autoimmun 38:J239–J244 [PubMed: 22261501]
22. Falany CN, Johnson MR, Barnes S, Diasio RB (1994) Glycine and taurine conjugation of bile acids by a single enzyme. Molecular cloning and expression of human liver bile acid CoA:amino acid N-acyltransferase. The J of Biol Chem 269:19375–19379 [PubMed: 8034703]
23. White BA, Fricke RJ, Hylemon PB (1982) 7 beta-Dehydroxylation of ursodeoxycholic acid by whole cells and cell extracts of the intestinal anaerobic bacterium, Eubacterium species V.P.I. 12708. J Lipid Res 23:145–153 [PubMed: 7057103]
24. Houten SM, Watanabe M, Auwerx J (2006) Endocrine functions of bile acids. The EMBO J 25:1419–1425 [PubMed: 16541101]
25. Yousef IM, Magnusson R, Price VM, Fisher MM (1973) Bile acid metabolism in mammals. V. Studies on the sex difference in the response of the isolated perfused rat liver to chenodeoxycholic acid. Can J Physiol Pharmacol 51:418–423 [PubMed: 4727809]
26. Kitada H, Miyata M, Nakamura T, Tozawa A, Honma W, Shimada M et al. (2003) Protective role of hydroxysteroid sulfotransferase in lithocholic acid-induced liver toxicity. The J of Biol Chem 278:17838–17844 [PubMed: 12637555]
27. Simon FR, Fortune J, Iwahashi M, Gartung C, Wolkoff A, Sutherland E (1996) Ethinyl estradiol cholestasis involves alterations in expression of liver sinusoidal transporters. The Am J of Physiol 271:G1043–G1052 [PubMed: 8997249]
28. Geier A, Wagner M, Dietrich CG, Trauner M (2007) Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. Biochim Biophys Acta 1773:283–308 [PubMed: 17291602]
29. Forker EL (1969) The effect of estrogen on bile formation in the rat. J Clin Invest 48:654–663 [PubMed: 5774104]
30. Davis RA, Kern F Jr, Showalter R, Sutherland E, Sinensky M, Simon FR (1978) Alterations of hepatic Na+, K+−atpase and bile flow by estrogen: effects on liver surface membrane lipid structure and function. Proc Natl Acad Sci U S A 75:4130–4134 [PubMed: 212735]
31. Elias E, Iqbal S, Knutton S, Hickey A, Coleman R (1983) Increased tight junction permeability: a possible mechanism of oestrogen cholestasis. Eur J Clin Investig 13:383–390 [PubMed: 6416852]
32. Jung D, Podvinec M, Meyer UA, Mangelsdorf DJ, Fried M, Meier PJ et al. (2002) Human organic anion transporting polypeptide 8 promoter is transactivated by the farnesoid X receptor/bile acid receptor. Gastroenterology 122:1954–1966 [PubMed: 12055601]
33. Aleksunes LM, Yeager RL, Wen X, Cui JY, Klaassen CD (2012) Repression of hepatobiliary transporters and differential regulation of classic and alternative bile acid pathways in mice during pregnancy. Toxicol Sci.130:257–268 [PubMed: 22903823]
34. Jeong S, Yoon M (2007) Inhibition of the actions of peroxisome proliferator-activated receptor alpha on obesity by estrogen. Obesity 15:1430–1440 [PubMed: 17557980]
35. Milona A, Owen BM, Cobbold JF, Willemsen EC, Cox IJ, Boudjelal M et al. (2010) Raised hepatic bile acid concentrations during pregnancy in mice are associated with reduced farnesoid X receptor function. Hepatology 52:1341–1349 [PubMed: 20842631]
36. Huang L, Smit JW, Meijer DK, Vore M (2000) Mrp2 is essential for estradiol-17beta(beta-D-glucuronide)-induced cholestasis in rats. Hepatology 32:66–72 [PubMed: 10869290]

37. Spivey JR, Bronk SF, Gores GJ (1993) Glycochenodeoxycholate-induced lethal hepatocellular injury in rat hepatocytes. Role of ATP depletion and cytosolic free calcium. J Clin Invest 92:17–24 [PubMed: 8325981]
38. Poupon RE, Balkau B, Eschwege E, Poupon R (1991) A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. N Engl J Med 324:1548–1554 [PubMed: 1674105]
39. Lamireau T, Zoltowska M, Levy E, Yousef I, Rosenbaum J, Tuchweber B et al. (2003) Effects of bile acids on biliary epithelial cells: proliferation, cytotoxicity, and cytokine secretion. Life Sci 72:1401–1411 [PubMed: 12527037]
40. Alpini G, Glaser SS, Ueno Y, Rodgers R, Phinizy JL, Francis H et al. (1999) Bile acid feeding induces cholangiocyte proliferation and secretion: evidence for bile acid-regulated ductal secretion. Gastroenterology 116:179–186 [PubMed: 9869616]
41. Alpini G, Ueno Y, Glaser SS, Marzioni M, Phinizy JL, Francis H et al. (2001) Bile acid feeding increased proliferative activity and apical bile acid transporter expression in both small and large rat cholangiocytes. Hepatology 34:868–876 [PubMed: 11679956]
42. Lazaridis KN, Pham L, Tietz P, Marinelli RA, DeGroen PC, Levine S et al. (1997) Rat cholangiocytes absorb bile acids at their apical domain via the ileal sodium-dependent bile acid transporter. J Clin Invest 100:2714–2721 [PubMed: 9389734]
43. Alpini G, Baiocchi L, Glaser S, Ueno Y, Marzioni M, Francis H et al. (2002) Ursodeoxycholate and tauroursodeoxycholate inhibit cholangiocyte growth and secretion of BDL rats through activation of PKC alpha. Hepatology 35:1041–1052 [PubMed: 11981754]
44. Alpini G, Glaser S, Robertson W, Phinizy JL, Rodgers RE, Caligiuri A et al. (1997) Bile acids stimulate proliferative and secretory events in large but not small cholangiocytes. The Am J of Physiol 273:G518–G529 [PubMed: 9277433]
45. Paumgartner G, Beuers U (2002) Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology 36:525–531 [PubMed: 12198643]
46. Deshmane SL, Kremlev S, Amini S, Sawaya BE (2009) Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 29:313–326 [PubMed: 19441883]
47. Demetris AJ, Lunz JG 3rd,Specht S, Nozaki I (2006) Biliary wound healing, ductular reactions, and IL-6/gp130 signaling in the development of liver disease. World J Gastroenterol 12:3512–3522 [PubMed: 16773708]
48. Benedetti A, Alvaro D, Bassotti C, Gigliozzi A, Ferretti G, La Rosa T et al. (1997) Cytotoxicity of bile salts against biliary epithelium: a study in isolated bile ductule fragments and isolated perfused rat liver. Hepatology 26:9–21 [PubMed: 9214446]
49. Heuman DM, Pandak WM, Hylemon PB, Vlahcevic ZR (1991) Conjugates of ursodeoxycholate protect against cytotoxicity of more hydrophobic bile salts: in vitro studies in rat hepatocytes and human erythrocytes. Hepatology 14:920–926 [PubMed: 1937396]
50. Miyazaki K, Nakayama F, Koga A (1984) Effect of chenodeoxycholic and ursodeoxycholic acids on isolated adult human hepatocytes. Dig Dis Sci 29:1123–1130 [PubMed: 6499631]
51. Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter D, and Shafritz DA et al. (1994) Bile acids. The liver: biology and pathobiology, third edition. New York: Raven 677–718
52. Verthelyi D (2001) Sex hormones as immunomodulators in health and disease. Int Immunopharmacol 1:983–993 [PubMed: 11407317]
53. Straub RH (2007) The complex role of estrogens in inflammation. Endocr Rev 28:521–574 [PubMed: 17640948]
54. Pillai S (2013) Rethinking mechanisms of autoimmune pathogenesis. J Autoimmun 45:97–103 [PubMed: 23809879]
55. Neuberger JM (1997) Primary biliary cirrhosis. Lancet 350:875–879 [PubMed: 9310614]
56. Ahlqvist J (1980) Can endocrine factors influence pathogenetic mechanisms in chronic active hepatitis (CAH) and primary biliary cirrhosis (PBC)? A hypothesis. Hepatogastroenterology 27:64–67 [PubMed: 6110625]
57. Floreani A, Titta M, Plebani M, Faggian D, Chiaramonte M, Naccarato R (1991) Sex hormone changes in post-menopausal women with primary biliary cirrhosis (PBC) and with cryptogenic chronic liver disease. Clin and Exp Obstet & Gynecol 18:229–234

58. Desmet VJ, van Eyken P, Roskams T (1998) Histopathology of vanishing bile duct diseases. Adv Clin Path 2:87–99
59. Desmet V, Roskams T, Van Eyken P (1995) Ductular reaction in the liver. Pathol Res Pract 191:513–524 [PubMed: 7479372]
60. Sampson LK, Vickers SM, Ying W, Phillips JO (1997) Tamoxifen-mediated growth inhibition of human cholangiocarcinoma. Cancer Res 57:1743–1749 [PubMed: 9135018]
61. Floreani A, Paternoster D, Mega A, Farinati F, Plebani M, Baldo V et al. (2002) Sex hormone profile and endometrial cancer risk in primary biliary cirrhosis: a case–control study. Eur J Obstet Gynecol Reprod Biol 103:154–157 [PubMed: 12069739]
62. Stellon AJ, Williams R (1986) Increased incidence of menstrual abnormalities and hysterectomy preceding primary biliary cirrhosis. Br Med J (Clin Res Ed) 293:297–298
63. Hodgson SF, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL (1985) Bone loss and reduced osteoblast function in primary biliary cirrhosis. Ann Intern Med 103:855–860 [PubMed: 4062087]
64. Olsson R, Mattsson LA, Obrant K, Mellstrom D (1999) Estrogen-progestogen therapy for low bone mineral density in primary biliary cirrhosis. Liver 19:188–192 [PubMed: 10395037]
65. Chen J, Robertson G, Field J, Liddle C, Farrell GC (1998) Effects of bile duct ligation on hepatic expression of female-specific CYP2C12 in male and female rats. Hepatology 28:624–630 [PubMed: 9731550]
66. Eagon PK, Porter LE, Francavilla A, DiLeo A, Van Thiel DH (1985) Estrogen and androgen receptors in liver: their role in liver disease and regeneration. Semin Liver Dis 5:59–69 [PubMed: 3885401]
67. Blum A, Cannon RO 3rd (1998) Effects of oestrogens and selective oestrogen receptor modulators on serum lipoproteins and vascular function. Curr Opin Lipidol 9:575–586 [PubMed: 9868594]
68. Eagon PK, Elm MS, Epley MJ, Shinozuka H, Rao KN (1996) Sex steroid metabolism and receptor status in hepatic hyperplasia and cancer in rats. Gastroenterology 110:1199–1207 [PubMed: 8613010]
69. Fisher B, Gunduz N, Saffer EA, Zheng S (1984) Relation of estrogen and its receptor to rat liver growth and regeneration. Cancer Res 44:2410–2415 [PubMed: 6722782]
70. Alvaro D, Alpini G, Onori P, Perego L, Svegliata Baroni G, Franchitto A et al. (2000) Estrogens stimulate proliferation of intrahepatic biliary epithelium in rats. Gastroenterology 119:1681–1691 [PubMed: 11113090]
71. Alvaro D, Alpini G, Onori P, Franchitto A, Glaser SS, Le Sage G et al. (2002) Alfa and beta estrogen receptors and the biliary tree. Mol Cell Endocrinol 193:105–108 [PubMed: 12161009]
72. Alvaro D, Alpini G, Onori P, Franchitto A, Glaser S, Le Sage G et al. (2002) Effect of ovariectomy on the proliferative capacity of intrahepatic rat cholangiocytes. Gastroenterology 123:336–344 [PubMed: 12105861]
73. Alvaro D, Invernizzi P, Onori P, Franchitto A, De Santis A, Crosignani A et al. (2004) Estrogen receptors in cholangiocytes and the progression of primary biliary cirrhosis. J Hepatol 41:905–912 [PubMed: 15645536]
74. Pierdominici M, Maselli A, Colasanti T, Giammarioli AM, Delunardo F, Vacirca D et al. (2010) Estrogen receptor profiles in human peripheral blood lymphocytes. Immunol Lett 132:79–85 [PubMed: 20542061]
75. Phiel KL, Henderson RA, Adelman SJ, Elloso MM (2005) Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. Immunol Lett 97:107–113 [PubMed: 15626482]
76. Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ et al. (1997) Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. Science 277:1508–1510 [PubMed: 9278514]
77. Gigliozzi A, Alpini G, Baroni GS, Marucci L, Metalli VD, Glaser SS et al. (2004) Nerve growth factor modulates the proliferative capacity of the intrahepatic biliary epithelium in experimental cholestasis. Gastroenterology 127:1198–1209 [PubMed: 15480997]

78. Alvaro D, Metalli VD, Alpini G, Onori P, Franchitto A, Barbaro B et al. (2005) The intrahepatic biliary epithelium is a target of the growth hormone/insulin-like growth factor 1 axis. J Hepatol 43:875–883 [PubMed: 16083987]
79. Gaudio E, Barbaro B, Alvaro D, Glaser S, Francis H, Ueno Y et al. (2006) Vascular endothelial growth factor stimulates rat cholangiocyte proliferation via an autocrine mechanism. Gastroenterology 130:1270–1282 [PubMed: 16618418]
80. You Z, Wang Q, Bian Z, Liu Y, Han X, Peng Y et al. (2012) The immunopathology of liver granulomas in primary biliary cirrhosis. J Autoimmun 39:216–221 [PubMed: 22727562]
81. Gershwin ME, Selmi C, Worman HJ, Gold EB, Watnik M, Utts J et al. (2005) Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. Hepatology 42:1194–1202 [PubMed: 16250040]
82. Corpechot C, Chretien Y, Chazouilleres O, Poupon R (2010) Demographic, lifestyle, medical and familial factors associated with primary biliary cirrhosis. J Hepatol 53:162–169 [PubMed: 20471130]
83. Compagnone NA, Mellon SH (2000) Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol 21:1–56 [PubMed: 10662535]
84. Micevych PE, Chaban V, Ogi J, Dewing P, Lu JK, Sinchak K (2007) Estradiol stimulates progesterone synthesis in hypothalamic astrocyte cultures. Endocrinology 148:782–789 [PubMed: 17095591]
85. Glaser S, DeMorrow S, Francis H, Ueno Y, Gaudio E, Vaculin S et al. (2008) Progesterone stimulates the proliferation of female and male cholangiocytes via autocrine/paracrine mechanisms. Am J Physiol Gastrointest Liver Physiol 295:G124–G136 [PubMed: 18511743]
86. Taffetani S, Glaser S, Francis H, DeMorrow S, Ueno Y, Alvaro D et al. (2007) Prolactin stimulates the proliferation of normal female cholangiocytes by differential regulation of Ca2+-dependent PKC PKC isoforms. BMC Physiol 7:6 [PubMed: 17640386]
87. Roskams T, Desmet V (1998) Ductular reaction and its diagnostic significance. Semin Diagn Pathol 15:259–269 [PubMed: 9845427]
88. Alvaro D, Onori P, Metalli VD, Svegliati-Baroni G, Folli F, Franchitto A et al. (2002) Intracellular pathways mediating estrogen-induced cholangiocyte proliferation in the rat. Hepatology 36:297–304 [PubMed: 12143037]
89. Robertson H, Ali S, McDonnell BJ, Burt AD, Kirby JA (2004) Chronic renal allograft dysfunction: the role of T cell-mediated tubular epithelial to mesenchymal cell transition. J Am Soc Nephrol 15:390–397 [PubMed: 14747385]
90. Burns WC, Kantharidis P, Thomas MC (2007) The role of tubular epithelial-mesenchymal transition in progressive kidney disease. Cells Tissues Organs 185:222–231 [PubMed: 17587828]
91. Diaz R, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS et al. (2008) Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. Hum Pathol 39:102–115 [PubMed: 17900655]
92. Omenetti A, Yang L, Li YX, McCall SJ, Jung Y, Sicklick JK et al. (2007) Hedgehog-mediated mesenchymal-epithelial interactions modulate hepatic response to bile duct ligation. Lab Invest 87:499–514 [PubMed: 17334411]
93. Ahrens EH Jr, Payne MA, Kunkel HG, Eisenmenger WJ, Blondheim SH (1950) Primary biliary cirrhosis. Medicine 29:299–364 [PubMed: 14796348]
94. Olsson R, Loof L, Wallerstedt S (1993) Pregnancy in patients with primary biliary cirrhosis—a case for dissuasion? The Swedish Internal Medicine Liver Club. Liver 13:316–318 [PubMed: 8295495]
95. Whelton MJ, Sherlock S (1968) Pregnancy in patients with hepatic cirrhosis. Manag and outcome Lancet 2:995–999
96. Nir A, Sorokin Y, Abramovici H, Theodor E (1989) Pregnancy and primary biliary cirrhosis. Int J Gynaecol Obstet 28:279–282 [PubMed: 2564359]
97. Rabinovitz M, Appasamy R, Finkelstein S (1995) Primary biliary cirrhosis diagnosed during pregnancy. Does it have a different outcome? Dig Dis Sci 40:571–574 [PubMed: 7895546]
98. Mack HC, Segar LF, Robinson AR, Wiseman ME, Moyer EZ (1953) Electrophoretic patterns of plasma proteins in pregnancy II. Pregnancy complicated by liver disease. Obstet Gynecol 1:204–211 [PubMed: 13037210]

99. Wong Kk GKL (1992) Pregnancy in primary biliary cirrhosis. Eur J Obstet Gynecol Reprod Biol 45:149–151 [PubMed: 1499849]
100. Ji H, Haring P, Kirkinen P, Saarikoski S (1995) Glucocorticoid treatment of primary biliary cirrhosis in a pregnant woman. Acta Obstet Gynecol Scand 74:654–656 [PubMed: 7660778]
101. Rudi J, Schonig T, Stremmel W (1996) Therapy with ursodeoxycholic acid in primary biliary cirrhosis in pregnancy. Z Gastroenterol 34:188–191 [PubMed: 8650973]
102. Goh SK, Gull SE, Alexander GJ (2001) Pregnancy in primary biliary cirrhosis complicated by portal hypertension: report of a case and review of the literature. BJOG 108:760–762 [PubMed: 11467706]
103. Korkut E, Kisacik B, Akcan Y, Belenli O, Bicik Z, Yucel O (2005) Two successive pregnancies after ursodeoxycholic acid therapy in a previously infertile woman with antimitochondrial antibody-negative primary biliary cirrhosis. Fertil Steril 83:761–763 [PubMed: 15749512]
104. Poupon R, Chretien Y, Chazouilleres O, Poupon RE (2005) Pregnancy in women with ursodeoxycholic acid-treated primary biliary cirrhosis. J Hepatol 42:418–419 [PubMed: 15710226]
105. Ohba K, Omagari K, Kusakari C, Kadokawa Y, Hayashida K, Takeshima F et al. (2005) Flare-up of autoimmune hepatitis after delivery in a patient with primary biliary cirrhosis: postpartum overlap syndrome of primary biliary cirrhosis and autoimmune hepatitis. Dig Dis Sci 50:201–206 [PubMed: 15712661]
106. Alallam A, Barth D, Heathcote EJ (2008) Role of plasmapheresis in the treatment of severe pruritus in pregnant patients with primary biliary cirrhosis: case reports. Can J Gastroenterol 22:505–507 [PubMed: 18478137]
107. Vitek L, Zelenkova M, Bruha R (2010) Safe use of ursodeoxycholic acid in a breast-feeding patient with primary biliary cirrhosis. Dig Liver Dis 42:911–912 [PubMed: 20619755]
108. Efe C, Ozaslan E, Purnak T (2011) Outcome of pregnancy in patients with autoimmune hepatitis/primary biliary cirrhosis overlap syndrome: a report of two cases. Clin Res Hepatol Gastroenterol 35:687–689 [PubMed: 21784721]
109. Matsubara S, Isoda N, Taniguchi N (2011) Jaundice as the first manifestation of primary biliary cirrhosis during pregnancy: measurement of portal vein blood flow. The J of Obstet and Gynaecol Res 37:963–964 [PubMed: 21651670]
110. Mitra S, Nayak PK, Padma A, Kurian G (2012) Successful pregnancy in autoimmune hepatitis/primary biliary cirrhosis overlap syndrome: a case report. Gastroenterol Res 5:208–210
111. Bogdanos DP, Smyk DS, Rigopoulou EI, Mytilinaiou MG, Heneghan MA, Selmi C et al. (2012) Twin studies in autoimmune disease: genetics, gender and environment. J Autoimmun 38:J156–J169 [PubMed: 22177232]
112. Riely CA (1999) Liver disease in the pregnant patient. Am J Gastroenterol 94:1728–1732 [PubMed: 10406228]
113. Reyes H (1997) Review: intrahepatic cholestasis. A puzzling disorder of pregnancy. J Gastroenterol Hepatol 12:211–216 [PubMed: 9142637]
114. Ropponen A, Sund R, Riikonen S, Ylikorkala O, Aittomaki K (2006) Intrahepatic cholestasis of pregnancy as an indicator of liver and biliary diseases: a population-based study. Hepatology 43:723–728 [PubMed: 16557542]
115. Marinaccio M, Pinto V, Sambati GS, Papandrea D, De Veteris M (1992) Uncomplicated pregnancy in patients with unrecognized primary biliary cirrhosis. Minerva Ginecol 44:273–276 [PubMed: 1608528]
116. Bacq Y, Zarka O, Brechot JF, Mariotte N, Vol S, Tichet J et al. (1996) Liver function tests in normal pregnancy: a prospective study of 103 pregnant women and 103 matched controls. Hepatology 23:1030–1034 [PubMed: 8621129]
117. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ, American Association for Study of Liver Diseases (2009) Primary biliary cirrhosis. Hepatology 50:50:291–308 [PubMed: 19554543]
118. Kam W, Clauser E, Kim YS, Kan YW, Rutter WJ (1985) Cloning, sequencing, and chromosomal localization of human term placental alkaline phosphatase cDNA. Proc Natl Acad Sci U S A 82:8715–8719 [PubMed: 3001717]

119. Dahlan Y, Smith L, Simmonds D, Jewell LD, Wanless I, Heathcote EJ et al. (2003) Pediatric-onset primary biliary cirrhosis. Gastroenterology 125:1476–1479 [PubMed: 14598264]
120. Baker ER (1985) Body weight and the initiation of puberty. Clin Obstet Gynecol 28:573–579 [PubMed: 4053451]
121. Floreani A, Ostuni PA, Ferrara F, Guido M (2006) Primary biliary cirrhosis: when and why does the disease develop? Dig Liver Dis 38:272–275 [PubMed: 16567309]
122. Shah R (2007) Pregnancy in a woman with primary biliary cirrhosis: does it affect foetal outcome and progression of maternal disease? gastroenteroltoday 17:94–96
123. Bianchi DW, Williams JM, Sullivan LM, Hanson FW, Klinger KW, Shuber AP (1997) PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. Am J Hum Genet 61:822–829 [PubMed: 9382092]
124. Nelson JL (1996) Maternal-fetal immunology and autoimmune disease: is some autoimmune disease auto-alloimmune or allo-autoimmune? Arthritis Rheum 39:191–194 [PubMed: 8849367]
125. Nelson JL, Furst DE, Maloney S, Gooley T, Evans PC, Smith A et al. (1998) Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. Lancet 351:559–562 [PubMed: 9492775]
126. Kobayashi H, Tamatani T, Tamura T, Kusafuka J, Yamataka A, Lane GJ et al. (2007) Maternal microchimerism in biliary atresia. J Pediatr Surg 42:987–991 [PubMed: 17560207]
127. Czaja AJ (1994) Chronic graft-versus-host disease and primary biliary cirrhosis: sorting the puzzle pieces. Lab Invest 70:589–592 [PubMed: 7910872]
128. Epstein O, Thomas HC, Sherlock S (1980) Primary biliary cirrhosis is a dry gland syndrome with features of chronic graft-versus-host disease. Lancet 1:1166–1168 [PubMed: 6103987]
129. McDonnell WM (1998) Is primary biliary cirrhosis a complication of pregnancy? Hepatol: off j of the Am Assoc for the Study of Liver Dis 28:593–594
130. Invernizzi P, De Andreis C, Sirchia SM, Battezzati PM, Zuin M, Rossella F et al. (2000) Blood fetal microchimerism in primary biliary cirrhosis. Clin Exp Immunol 122:418–422 [PubMed: 11122249]
131. Tanaka A (1999) Fetal microchimerism alone does not contribute to the induction of primary biliary cirrhosis. Hepatology 30:833–838 [PubMed: 10498630]
132. Corpechot C, Barbu V, Chazouilleres O, Poupon R (2000) Fetal microchimerism in primary biliary cirrhosis. J Hepatol 33:696–700 [PubMed: 11097475]
133. Fanning PA, Jonsson JR, Clouston AD, Edwards-Smith C, Balderson GA, Macdonald GA et al. (2000) Detection of male DNA in the liver of female patients with primary biliary cirrhosis. J Hepatol 33:690–695 [PubMed: 11097474]
134. Stevens AM, McDonnell WM, Mullarkey ME, Pang JM, Leisenring W, Nelson JL (2004) Liver biopsies from human females contain male hepatocytes in the absence of transplantation. Lab Invest 84:1603–1609 [PubMed: 15502859]
135. Palmer JM, Kirby JA, Jones DE (2002) The immunology of primary biliary cirrhosis: the end of the beginning? Clin Exp Immunol 129:191–197 [PubMed: 12165073]
136. Bianchi I, Lleo A, Gershwin ME, Invernizzi P (2012) The X chromosome and immune associated genes. J Autoimmun 38:J187–J192 [PubMed: 22178198]
137. Lu Q (2013) The critical importance of epigenetics in autoimmunity. J Autoimmun 41:1–5 [PubMed: 23375849]
138. Thellin O, Coumans B, Zorzi W, Igout A, Heinen E (2000) Tolerance to the foeto-placental 'graft': ten ways to support a child for nine months. Curr Opin Immunol 12:731–737 [PubMed: 11102780]
139. Moreau P, Lefebvre S, Gourand L, Dausset J, Carosella ED, Paul P (1998) Specific binding of nuclear factors to the HLA-G gene promoter correlates with a lack of HLA-G transcripts in first trimester human fetal liver. Hum Immunol 59:751–757 [PubMed: 9831130]
140. Thellin O, Heinen E (2003) Pregnancy and the immune system: between tolerance and rejection. Toxicology 185:179–184 [PubMed: 12581692]

141. Giltay EJ, Fonk JC, von Blomberg BM, Drexhage HA, Schalkwijk C, Gooren LJ (2000) In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. J Clin Endocrinol Metab 85:1648–1657 [PubMed: 10770211]
142. Watanabe M, Iwatani Y, Kaneda T, Hidaka Y, Mitsuda N, Morimoto Y et al. (1997) Changes in T, B, and NK lymphocyte subsets during and after normal pregnancy. Am J Reprod Immunol 37:368–377 [PubMed: 9196795]
143. Kuhnert M, Strohmeier R, Stegmuller M, Halberstadt E (1998) Changes in lymphocyte subsets during normal pregnancy. Eur J Obstet Gynecol Reprod Biol 76:147–151 [PubMed: 9481564]
144. Veenstra van Nieuwenhoven ALV, Bouman A, Moes H, Heineman MJ, de Leij LF, Santema J et al. (2002) Cytokine production in natural killer cells and lymphocytes in pregnant women compared with women in the follicular phase of the ovarian cycle. Fertil Steril 77:1032–1037 [PubMed: 12009363]
145. Kanik KS, Wilder RL (2000) Hormonal alterations in rheumatoid arthritis, including the effects of pregnancy. Rheum Dis Clin North Am 26:805–823 [PubMed: 11084945]
146. Munoz-Valle JF, Vazquez-Del Mercado M, Garcia-Iglesias T, Orozco-Barocio G, Bernard-Medina G, Martinez-Bonilla G et al. (2003) T(H)1/T(H)2 cytokine profile, metalloprotease-9 activity and hormonal status in pregnant rheumatoid arthritis and systemic lupus erythematosus patients. Clin Exp Immunol 131:377–384 [PubMed: 12562402]
147. Groer MW, El-Badri N, Djeu J, Williams SN, Kane B, Szekeres K (2013) Suppression of natural killer cell cytotoxicity in postpartum women: time course and potential mechanisms. Biol Res for Nur 16:320–326
148. Chuang YH, Lian ZX, Tsuneyama K, Chiang BL, Ansari AA, Coppel RL et al. (2006) Increased killing activity and decreased cytokine production in NK cells in patients with primary biliary cirrhosis. J Autoimmun 26:232–240 [PubMed: 16730427]
149. Matsuda JL, Mallevaey T, Scott-Browne J, Gapin L (2008) CD1d-restricted iNKT cells, the 'Swiss-army knife' of the immune system. Curr Opin Immunol 20:358–368 [PubMed: 18501573]
150. Harada K, Van de Water J, Leung PS, Coppel RL, Ansari A, Nakanuma Y et al. (1997) In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. Hepatology 25:791–796 [PubMed: 9096578]
151. Kita H, Naidenko OV, Kronenberg M, Ansari AA, Rogers P, He XS et al. (2002) Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. Gastroenterology 123:1031–1043 [PubMed: 12360465]
152. Tsuneyama K, Yasoshima M, Harada K, Hiramatsu K, Gershwin ME, Nakanuma Y (1998) Increased CD1d expression on small bile duct epithelium and epithelioid granuloma in livers in primary biliary cirrhosis. Hepatology 28:620–623 [PubMed: 9731549]
153. Chuang YH, Lian ZX, Yang GX, Shu SA, Moritoki Y, Ridgway WM et al. (2008) Natural killer T cells exacerbate liver injury in a transforming growth factor beta receptor II dominant-negative mouse model of primary biliary cirrhosis. Hepatology 47:571–580 [PubMed: 18098320]
154. Aso-Ishimoto Y, Yamagiwa S, Ichida T, Miyakawa R, Tomiyama C, Sato Y et al. (2014) Increased activated natural killer T cells in the liver of patients with advanced stage primary biliary cirrhosis. Biomed Res 35:161–169 [PubMed: 24759184]
155. Nakaya M, Tachibana H, Yamada K (2006) Effect of estrogens on the interferon-gamma producing cell population of mouse splenocytes. Biosci Biotechnol Biochem 70:47–53 [PubMed: 16428820]
156. Southcombe J, Redman C, Sargent I (2010) Peripheral blood invariant natural killer T cells throughout pregnancy and in preeclamptic women. J Reprod Immunol 87:52–59 [PubMed: 20850184]
157. Bouman A, Heineman MJ, Faas MM (2005) Sex hormones and the immune response in humans. Hum Reprod Update 11:411–423 [PubMed: 15817524]
158. Luppi P, Haluszczak C, Betters D, Richard CA, Trucco M, DeLoia JA (2002) Monocytes are progressively activated in the circulation of pregnant women. J Leukoc Biol 72:874–884 [PubMed: 12429709]

159. Mao TK, Lian ZX, Selmi C, Ichiki Y, Ashwood P, Ansari AA et al. (2005) Altered monocyte responses to defined TLR ligands in patients with primary biliary cirrhosis. Hepatology 42:802–808 [PubMed: 16175622]

160. Gentile T, Llambias P, Dokmetjian J, Margni RA (1998) Effect of pregnancy and placental factors on the quality of humoral immune response. Immunol Lett 62:151–157 [PubMed: 9698113]

161. Miller EC, Abel W (1984) Changes in the immunoglobulins IgG, IgA and IgM in pregnancy and the puerperium. Zentralbl Gynakol 106:1084–1091 [PubMed: 6495924]

162. Lleo A, Liao J, Invernizzi P, Zhao M, Bernuzzi F, Ma L et al. (2012) Immunoglobulin M levels inversely correlate with CD40 ligand promoter methylation in patients with primary biliary cirrhosis. Hepatology 55:153–160 [PubMed: 21898485]

163. Scaglione BJ, Salerno E, Gala K, Pan M, Langer JA, Mostowski HS et al. (2009) Regulatory T cells as central regulators of both autoimmunity and B cell malignancy in New Zealand Black mice. J Autoimmun 32:14–23 [PubMed: 19022624]

164. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S (2004) Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod 10:347–353 [PubMed: 14997000]

165. Heikkinen J, Mottonen M, Alanen A, Lassila O (2004) Phenotypic characterization of regulatory T cells in the human decidua. Clin Exp Immunol 136:373–378 [PubMed: 15086404]

166. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT (2004) Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology 112:38–43 [PubMed: 15096182]

167. Tilburgs T, Roelen DL, van der Mast BJ, van Schip JJ, Kleijburg C, de Groot-Swings GM et al. (2006) Differential distribution of CD4(+)CD25(bright) and CD8(+)CD28(−) T-cells in decidua and maternal blood during human pregnancy. Placenta 27 Suppl A:S47–53 [PubMed: 16442616]

168. Mjosberg J, Berg G, Ernerudh J, Ekerfelt C (2007) CD4+ CD25+ regulatory T cells in human pregnancy: development of a Treg-MLC-ELISPOT suppression assay and indications of paternal specific Tregs. Immunology 120:456–466 [PubMed: 17229266]

169. Jordan MA, Baxter AG (2008) The genetics of immunoregulatory T cells. J Autoimmun 31:237–244 [PubMed: 18550334]

170. Forger F, Marcoli N, Gadola S, Moller B, Villiger PM, Ostensen M (2008) Pregnancy induces numerical and functional changes of CD4 + CD25 high regulatory T cells in patients with rheumatoid arthritis. Ann Rheum Dis 67:984–990 [PubMed: 17971458]

171. Wegmann TG, Lin H, Guilbert L, Mosmann TR (1993) Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 14:353–356 [PubMed: 8363725]

172. Laird SM, Tuckerman EM, Cork BA, Linjawi S, Blakemore AI, Li TC (2003) A review of immune cells and molecules in women with recurrent miscarriage. Hum Reprod Update 9:163–174 [PubMed: 12751778]

173. Chaouat G, Ledee-Bataille N, Dubanchet S, Zourbas S, Sandra O, Martal J (2004) TH1/TH2 paradigm in pregnancy: paradigm lost? Cytokines in pregnancy/early abortion: reexamining the TH1/TH2 paradigm. Int Arch Allergy Immunol 134:93–119 [PubMed: 15153791]

174. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG (1993) Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol 151:4562–4573 [PubMed: 8409418]

175. Choi BC, Polgar K, Xiao L, Hill JA (2000) Progesterone inhibits in-vitro embryotoxic Th1 cytokine production to trophoblast in women with recurrent pregnancy loss. Hum Reprod 15(Suppl 1):46–59 [PubMed: 10928418]

176. Bernton EW, Meltzer MS, Holaday JW (1988) Suppression of mac rophage activation and T-lymphocyte function in hypoprolactinemic mice. Science 239:401–404 [PubMed: 3122324]

177. Andrews ZB (2005) Neuroendocrine regulation of prolactin secretion during late pregnancy: easing the transition into lactation. J Neuroendocrinol 17:466–473 [PubMed: 15946165]

178. Matalka KZ (2003) Prolactin enhances production of interferon-gamma, interleukin-12, and interleukin-10, but not of tumor necrosis factor-alpha, in a stimulus-specific manner. Cytokine 21:187–194 [PubMed: 12788307]

179. Matera L, Mori M (2000) Cooperation of pituitary hormone prolactin with interleukin-2 and interleukin-12 on production of interferon-gamma by natural killer and T cells. Ann N Y Acad Sci 917:505–513 [PubMed: 11268378]
180. Van de Water J, Ansari A, Prindiville T, Coppel RL, Ricalton N, Kotzin BL et al. (1995) Heterogeneity of autoreactive T cell clones specific for the E2 component of the pyruvate dehydrogenase complex in primary biliary cirrhosis. J Exp Med 181:723–733 [PubMed: 7836925]
181. Van de Water J, Ansari AA, Surh CD, Coppel R, Roche T, Bonkovsky H et al. (1991) Evidence for the targeting by 2-oxo-dehydrogenase enzymes in the T cell response of primary biliary cirrhosis. J Immunol 146:89–94 [PubMed: 1984455]
182. Lohr H, Fleischer B, Gerken G, Yeaman SJ, Buschenfelde KH M z, Manns M (1993) Autoreactive liver-infiltrating T cells in primary biliary cirrhosis recognize inner mitochondrial epitopes and the pyruvate dehydrogenase complex. J Hepatol 18:322–327 [PubMed: 7693799]
183. Jones DE, Palmer JM, James OF, Yeaman SJ, Bassendine MF, Diamond AG (1995) T-cell responses to the components of pyruvate dehydrogenase complex in primary biliary cirrhosis. Hepatology 21:995–1002 [PubMed: 7705811]
184. Yamada G, Hyodo I, Tobe K, Mizuno M, Nishihara T, Kobayashi T et al. (1986) Ultrastructural immunocytochemical analysis of lymphocytes infiltrating bile duct epithelia in primary biliary cirrhosis. Hepatology 6:385–391 [PubMed: 2940162]
185. Kita H (2007) Autoreactive CD8-specific T-cell response in primary biliary cirrhosis. Hepatol Res 37(Suppl 3):S402–S405 [PubMed: 17931194]
186. Nagano T, Yamamoto K, Matsumoto S, Okamoto R, Tagashira M, Ibuki N et al. (1999) Cytokine profile in the liver of primary biliary cirrhosis. J Clin Immunol 19:422–427 [PubMed: 10634216]
187. Itoh S, Matsuzaki Y, Kimura T, Ikegami T, Shoda J, Fujiwara M et al. (2000) Cytokine profile of liver-infiltrating CD4+ T cells separated from murine primary biliary cirrhosis-like hepatic lesions induced by graft-versus-host reaction. J Gastroenterol Hepatol 15:443–451 [PubMed: 10824891]
188. Steinman L (2007) A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 13:139–145 [PubMed: 17290272]
189. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM et al. (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6:1123–1132 [PubMed: 16200070]
190. Acosta-Rodriguez Ev NGLASF (2007) Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol 8:942–949 [PubMed: 17676045]
191. Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas De St Groth B et al. (2009) Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol 183:7023–7030 [PubMed: 19915051]
192. Saito S, Nakashima A, Shima T, Ito M (2010) Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol 63:601–610 [PubMed: 20455873]
193. Rong G, Zhou Y, Xiong Y, Zhou L, Geng H, Jiang T et al. (2009) Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population. Clin Exp Immunol 156:217–225 [PubMed: 19302244]
194. Tanaka H, Yang GX, Iwakoshi N, Knechtle SJ, Kawata K, Tsuneyama K et al. (2013) Anti-CD40 ligand monoclonal antibody delays the progression of murine autoimmune cholangitis. Clin Exp Immunol 174:364–371 [PubMed: 23981074]
195. Kawata K, Yang GX, Ando Y, Tanaka H, Zhang W, Kobayashi Y et al. (2013) Clonality, activated antigen-specific CD8(+) T cells, and development of autoimmune cholangitis in dnTGFbetaRII mice. Hepatology 58:1094–1104 [PubMed: 23532950]
196. Dhirapong A, Yang GX, Nadler S, Zhang W, Tsuneyama K, Leung P et al. (2013) Therapeutic effect of cytotoxic T lymphocyte antigen 4/immunoglobulin on a murine model of primary biliary cirrhosis. Hepatology 57:708–715 [PubMed: 22996325]

197. Tsuda M, Zhang W, Yang GX, Tsuneyama K, Ando Y, Kawata K et al. (2013) Deletion of interleukin (IL)-12p35 induces liver fibrosis in dominant-negative TGFbeta receptor type II mice. Hepatology 57:806–816 [PubMed: 22576253]

198. Ando Y, Yang GX, Tsuda M, Kawata K, Zhang W, Nakajima T et al. (2012) The immunobiology of colitis and cholangitis in interleukin-23p19 and interleukin-17A deleted dominant negative form of transforming growth factor beta receptor type II mice. Hepatology 56:1418–1426 [PubMed: 22532156]

199. Yang CY, Ma X, Tsuneyama K, Huang S, Takahashi T, Chalasani NP et al. (2014) IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy. Hepatology 59:1944–1953 [PubMed: 24375552]

200. Zhang J, Zhang W, Leung PS, Bowlus CL, Dhaliwal S, Coppel RL et al. (2014) Ongoing activation of autoantigen-specific b cells in primary biliary cirrhosis. Hepatology

201. Lleo A, Zhang W, McDonald WH, Seeley EH, Leung PS, Coppel RL et al. (2014) Shotgun proteomics: identification of unique protein profiles of apoptotic bodies from biliary epithelial cells. Hepatology

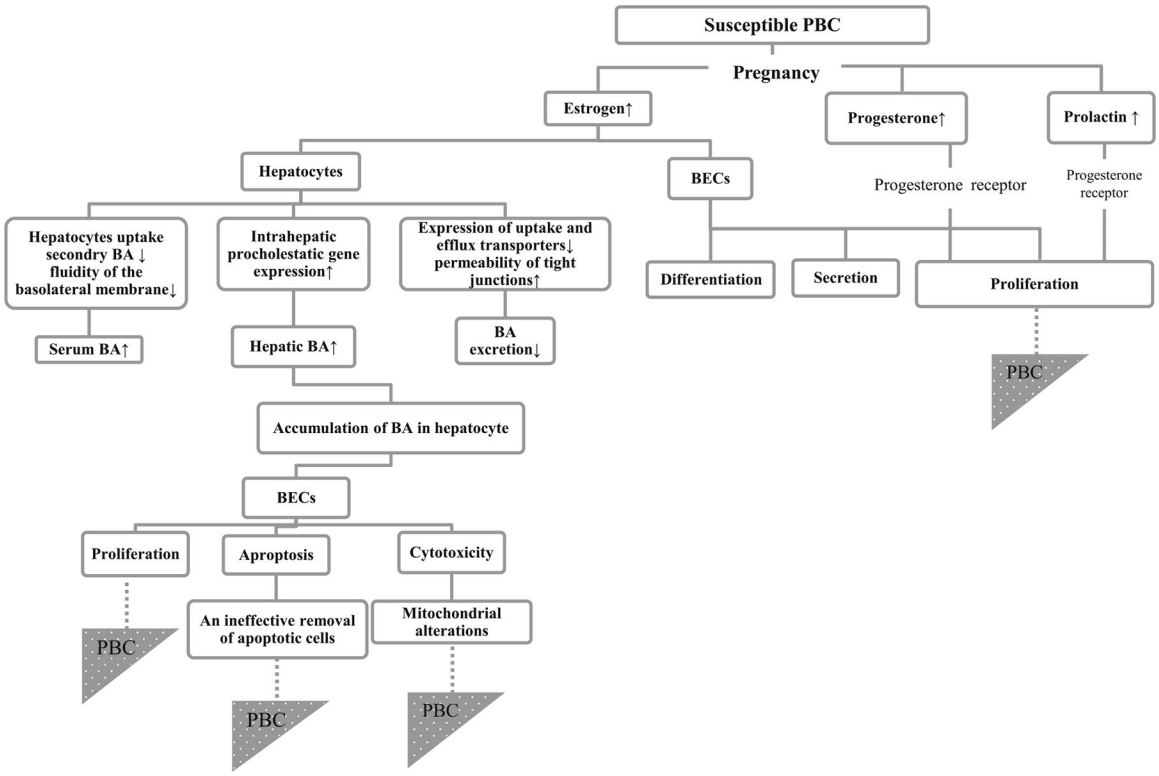

Fig. 1.

Estrogen, progesterone, and prolactin interact with BECs and hepatocytes during pregnancy. Pregnancy creates a susceptible environment for the ontogeny of PBC under certain genetic and environmental conditions. During gestation, the drastic elevation in female hormones, estrogen, progesterone, and, prolactin, affect BA metabolism and BEC proliferation resulting in accumulation of BA in hepatocyte and apoptotic cell death in BEC

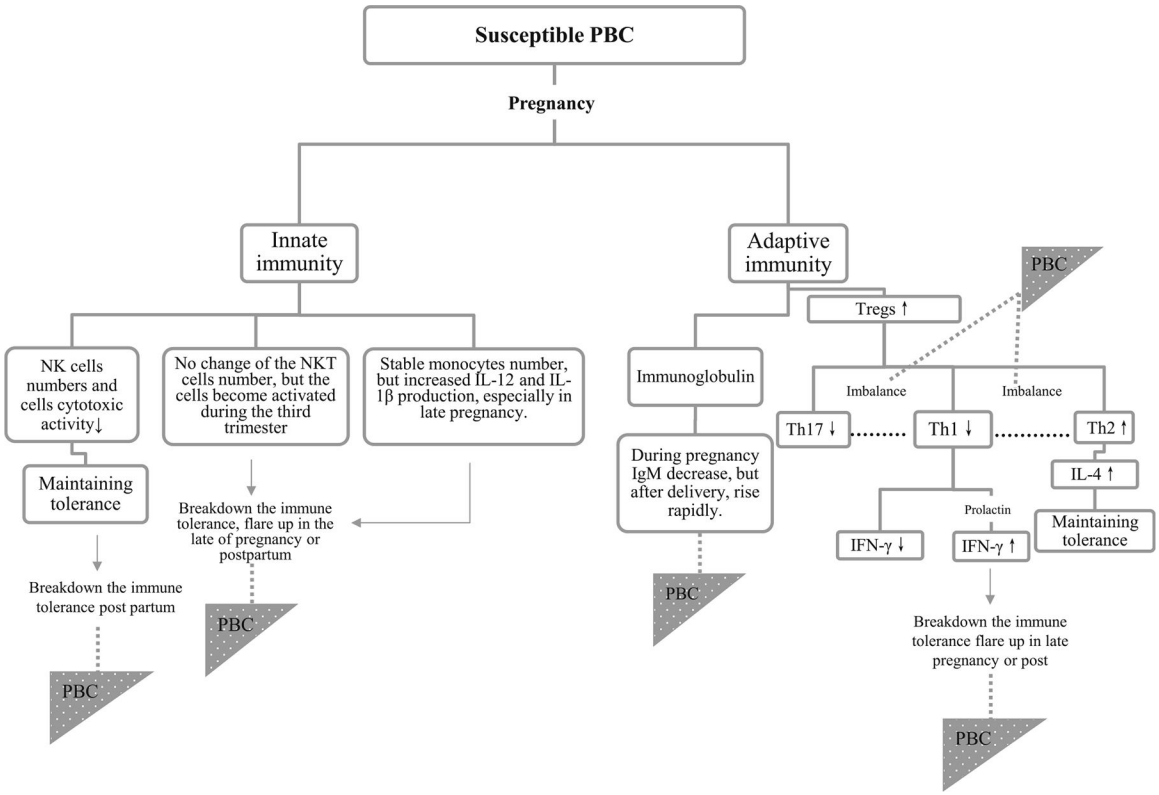

Fig. 2.

Maintaining immune tolerance during pregnancy. Fine-tuning maternal innate and adaptive immune response occurs to maintain fetal tolerance, which may create an environment for the ontogeny of PBC in susceptible women

Table 1

Characteristic of 607 pregnancies from 259 patients with PBC

	Case report[a]	Retrospective cohort study [2]	Case-control study [4]
Total number of PBC			
Patients/pregnancies	41/50	32/50	186/507
Median or mean age (range) (years)	34 (20–47), 33.6±5.3	32 (23–44)	29.5±5.6
Onset of pregnancy			
Before diagnosis of PBC	7 (14.0 %)	17 (53.0 %)	499 (98.4 %)
After diagnosis of PBC	43 (86.0 %)	15 (47.0 %)	8 (1.6 %)
Fibrosis stage	34	31	8
0-II		27/31 (87.1 %)	–
I	1/34	–	4/8 (50.0 %)
III	2/34	–	–
I—III	8/34	–	–
II	2/34	–	–
II–III	1/34	–	–
II or III	9/34	–	–
III	6/34	–	1/8 (12.5 %)
III–IV	–	4/3 (12.9 %)	
IV	–	–	3/8 (37.5 %)
OS (PBC/AIH)	5/34	–	–
PBC activity during pregnancy		–	–
Stable	27/50 (54.0 %)	20/50 (40.0 %)	–
Flare	20/50 (40.0 %)	30/50 (60.0 %)	–
Improvement	3/50 (6.0 %)	–	–
PBC activity postpartum		–	–
Stable	16/50 (32.0 %)	16/23 (69.6 %)	–
Flare	17/50 (34.0 %)	7/23 (30.4 %)	–
Improvement	2/50 (4.0 %)	–	–
Deterioration	8/50 (16.0 %)	–	–
NA	7/50 (14.0 %)	–	–

	Case report[a]	Retrospective cohort study [2]	Case-control study [4]
Pruritus	18/50 (36.0 %)	16/50 (32.0 %)	15/507 (3.0 %)
Jaundice	14/50 (28.0 %)	–	–
Others—nausea, epistaxis, fatigue, preeclampsia, night blindness, bone pain	4/50 (8.0 %)	–	–
UDCA therapy during pregnancy	–	–	–
Taking UDCA	32 (64.0 %)	–	–
No taking UDCA	2 (4.0 %)	–	–
No information	16 (32.0 %)	–	–
Fetal outcome		–	–
Live	42/50 (84.0 %)	39/50 (78.0 %)	409/507 (80.7 %)
Preterm	7		–
Spontaneous abortion	6 (12.0 %)	20/50 (40 %)	87/507 (17.2 %)
Stillbirth	2 (4.0 %)	1/50 (2.0 %)	–
Voluntary interruptions of pregnancy	0	0	11/507 (2.1 %)
Gender of the fetus	–	–	–
Female	7	–	–
Male	9	–	–
No information	34	–	–

[a]Refs. [4, 93–110, 122]