Current Trends in Immunology
Vol. 14, 2013

Review

Viruses as triggers in the pathogenesis of infantile biliary atresia

Toshiharu Hayashi[1,*], Tomomi Nakashima[1] and Takuya Mizuno[2]

[1]Laboratory of Veterinary Pathology and [2]Veterinary Internal Medicine, Joint Faculty of Veterinary Medicine, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

ABSTRACT

Biliary atresia (BA) in infantile is unique and an inflammatory cholangiopathy that culminates in a progressive sclerosis of the extrahepatic biliary tree, resulting in cholestasis by complete obliteration of the lumen of the bile ducts. Although etiologies of biliary atresia are not completely determined, several factors have been proposed. Among them, triggering roles of viral infection of the primary perinatal hepatobiliary epithelium which leads to autoimmune-mediated bile duct injury with destruction of self-tolerance have been suggested. Thereafter, sclerosis of the extrahepatic biliary tree and intrahepatic bile ducts may develop by continued injury. This brief article focuses mainly on the possibility of viruses such as rotavirus, reovirus and cytomegalovirus in a triggering role in the pathogenesis of BA. Also viruses as precipitator and underlying mechanisms in the development of autoimmunity to bile ducts and sclerosis will be discussed.

KEYWORDS: biliary atresia, virus, etiology, autoimmune, fibrosis, pathogenesis

INTRODUCTION

Biliary atresia (BA), which is the most common cause of liver transplantation in children, is a progressive, inflammatory and sclerosing process that leads to the complete obliteration of the bile ducts at any point from the porta hepatis to the duodenum with replacement by fibrous remnants and resulting in biliary cirrhosis [1].

The etiopathogenesis of BA has not yet been elucidated. The most widely mentioned hypotheses (i.e., infectious, congenital, toxic, and immune-mediated) are often considered as independent mechanisms leading to BA. It has been suggested that the pathogenesis of this disease is orchestrated by a complex interplay of inflammatory, fibrogenic, and morphogenetic factors. Landing [2] suggested that acquired BA could be caused by a virus infection. Mack [3] reviewed the outline of pathogenesis of BA as follows. The bile duct damage is initiated by a virus infection followed by the release of altered "self" antigens that activate bile duct-specific autoreactive T cells, resulting in a chronic, inflammatory fibrosclerosing injury of the bile ducts. Using cytomegalovirus (CMV) infection in a mouse model of BA, Brindley *et al.* [4] suggested that pathogenic mechanisms of autoimmunity in BA include "bystander activation", molecular mimicry, and loss of inhibition of autoimmunity due to defects in regulatory T cells (Tregs). They identified autoreactive T cells specific to bile duct epithelium, which contribute to bile duct injury. Also, the rotavirus induced mouse model of BA showed this virus-induced, autoimmune-mediated pathway of bile duct injury [5-7]. In addition, we have recently reported reovirus-type 2 (Reo-2)-triggered cholangitis in a mouse model, which may have been induced by autoimmune reaction against duct epithelium [8]. Furthermore, a role for humoral autoimmunity in mouse and human BA was identified based

*Corresponding author: hayasi@yamaguchi-u.ac.jp

on the detection of high levels of α-enolase autoantibodies [9].

Although the etiology and pathogenesis of BA are largely unknown and segmental as described above, there are accumulating evidences that viruses may play a role in triggering and/or as a candidate in the pathogenesis of BA (Fig. 1). This article focuses mainly on the virus-triggered autoimmunity to bile ducts including the role of virus as precipitator together with underlying mechanisms in BA. Also, the factors which may permit the autoimmune reaction leading to fibroscrelosing cholangitis will be discussed.

Roles of viruses as trigger

This section introduces viruses suspected as causative agents, especially in roles of induction of immune-mediated cholangitis. Viruses may contribute to the development of cholangitis directly and indirectly. Although viruses can induce autoimmunity via different mechanisms, primarily destruction of bile duct may occur by the multiplication of direct duct epithelial tropic infection, resulting in the release not only of virus particles but also auto-duct antigens. Three viruses, such as rotavirus, cytomegalovirus (CMV) and reovirus (Reo), have received increasing attention as possible inciters of an immune-mediated injury to the biliary tree.

BA patients at diagnosis showed infection of Reo, rotavirus and CMV associated with the disease onset [1]. On the other hand, it has also been reported that no virus isolation was observed in humans with BA [3]. To address these conflicting results, Brindley *et al.* [4] took a different approach and demonstrated a liver T-cell response encompassing resident virus-specific memory T cells, based on the argument that the memory response is long-lasting and would be present even in the setting of viral clearance. Also, virus is cleared within the first 2 weeks in the rotavirus induced mouse model of BA [5-7], despite progression of inflammation and bile duct obstruction. These considerations support the idea that viruses may play a role as inciters in BA.

Feng *et al.* [10] point out that rotavirus infection is an initiator in the pathogenesis of experimental BA through the induction of increased nuclear factor-kappa β and abnormal activation of the osteopontin inflammation pathway as mentioned below. Rotavirus nonstructural protein 4 (NSP4) serves as an important immunogen, viral protein 7 (VP7) is necessary in rotavirus maturation and viral protein 4 (VP4) is a virulence determiner. Primary cultured extrahepatic biliary epithelia showed that NSP4 silencing decreased the levels of VP7 and VP4, reduced viral particles and decreased cytopathic effects. NSP4-positive cells had a strong positive expression of integrin subunit α2. Silencing of VP7 or VP4 partially decreased epithelial injury. Animal experiments indicated that, after NSP4 silencing, mouse pups had lower incidence of BA than after VP7 or VP4 silencing. However, 33.3% of VP4-silenced pups suffered BA and 50% of pups suffered biliary injury after VP7 silencing. Hepatic injury was decreased after NSP4 or VP4 silencing. Neither VP4 nor VP7 were detected in the biliary ducts after NSP4 silencing. All together, NSP4 silencing down-regulates VP7 and VP4, resulting in decreased incidence of BA.

As another example, two types of duct destruction such as necrosis and apoptosis may be induced by Reo-2 infection during initiation phase [8]. Attachment of reovirus to target cells is mediated through the filamentous, trimer reovirus signa1 protein (σ) 1, which is a serotype-independent receptor, and epithelial cell necrosis may occur after multiplication in those cells. At the same time, reovirus-induced apoptosis will be induced by the release of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) from infected cells and the activation of TRAIL-associated death receptors (DRs), DR4 and DR5, leading to apoptosis.

Mechanisms by which viruses trigger immune-mediated injury of bile duct epithelium

This section introduces the mechanisms of virus-induced immunologic injury to biliary ducts based on well-established ideas in virus-induced autoimmune diseases reviewed by Kivity *et al.* [11].

In general post-infection autoimmunity can be induced by multiple mechanisms as follows:

I. Molecular mimicry is perhaps the most likely mechanism by which infections induce autoimmunity. It is characterized by cross-reactivity between

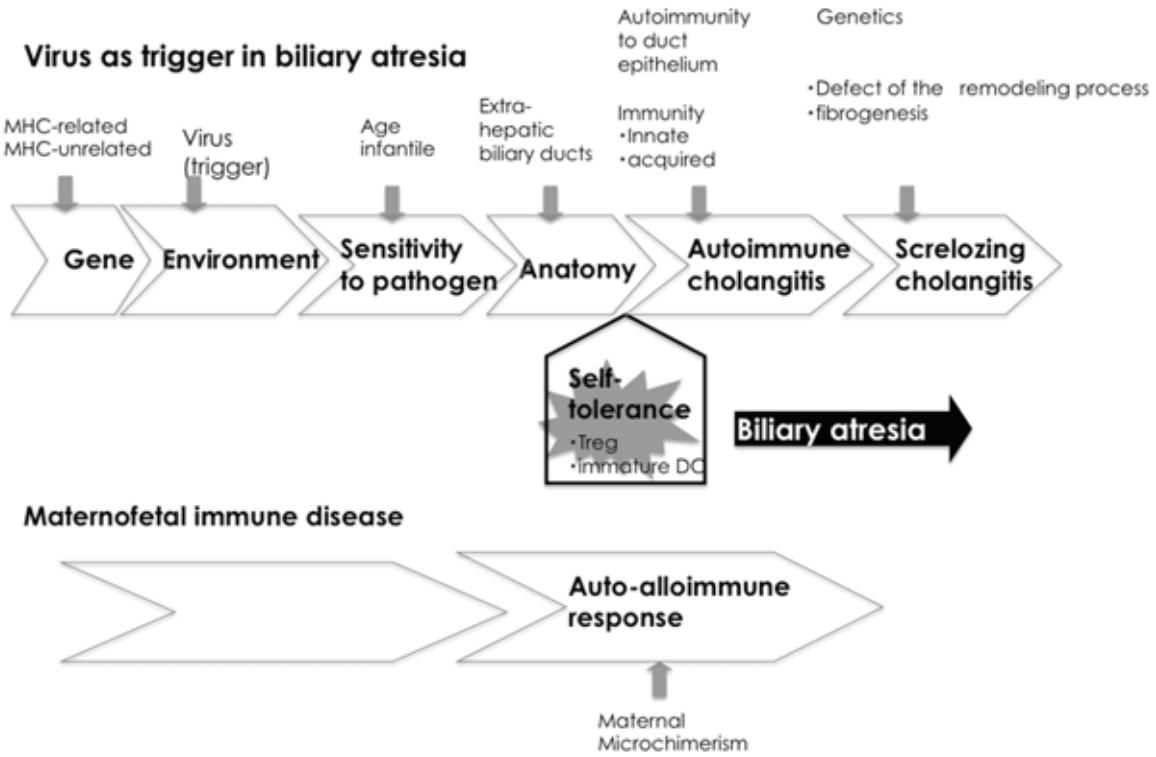

Fig. 1. Combined effects of several factors, which may be responsible for the development of BA in virus-triggered immune disease (upper), and maternofetal immune disease (lower) in the pathogenesis of BA (hypothesis).

epitopes (i.e. protein, carbohydrate or DNA) shared by the pathogen and the host. Molecular mimicry mechanism may play a role in pathogenesis of BA [9], although it is not conclusive. The criteria for this mechanism include: (i) the pathogen must be associated with the onset of the autoimmune diseases; (ii) the pathogen must provoke an immune response that cross-reacts with host antigens; (iii) the shared epitopes (auto-antigens) should induce the disease in an animal model (active immunization); (iv) autoreactive T cells or auto-antibodies should induce the disease in animal models (passive immunization). However, these criteria are not proven in BA except for (i).

II. Epitope spreading is a diversification of epitope specificity from a dominant epitope to subdominant (cryptic) epitopes. The switch from dominant to cryptic epitopes begins with molecular mimicry sharing to the dominant epitope, followed by protein processing and antigen presentation, resulting in an autoimmune response directed at a neo-epitope. Sokol and Mack [1] suggest this possibility in their review.

III. Bystander activation occurs when virally induced tissue damage causes the release of sequestered antigen which activates autoreactive lymphocytes that were not directly involved in the initial reactivity to the virus. In addition, virally infected antigen presenting cells (APCs) are able to activate macrophages which perturb pre-primed autoreactive T cells in a bystander manner. Virus-specific T cells might initiate bystander activation upon recognition of virally infected cells and by releasing cytotoxic granules and cytokines. An inflammatory microenvironment is created, with cytokines such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, active oxygen species and other molecules (e.g., perforin and granzyme), that can lead to bystander killing of neighboring (uninfected) cells and bystander activation of an autoimmune response.

IV. Prolonged infectivity with viruses, viral proteins or viral genome can lead to autoimmunity via constant activation of the immune response. After such polyclonal activation and proliferation of B cells, sometimes a monospecific proliferation

can emerge, accompanied by circulating immune complexes and eventually damage to self-tissues. Hypothetical Reo-2-triggered autoimmune cholangitis is shown in Fig. 2 together with the participation of NK cell obtained from rotavirus model [7].

Roles of viruses as a participator

This section introduces the role of viruses as a participator besides their triggering role, although there are no reports from this point of view. The "one organism-one disease" paradigm that is central to Koch's postulates might not invariably apply to microbially induced autoimmune disease and the "fertile field hypothesis" is proposed by von Herrath *et al.* [12]. Kivity *et al.* [11] supported this idea that the 'burden of infections' from childhood rather than a single infection may be responsible for the induction of autoimmunity. This suggests that a viral infection alone might not be able to cause clinical autoimmunity but

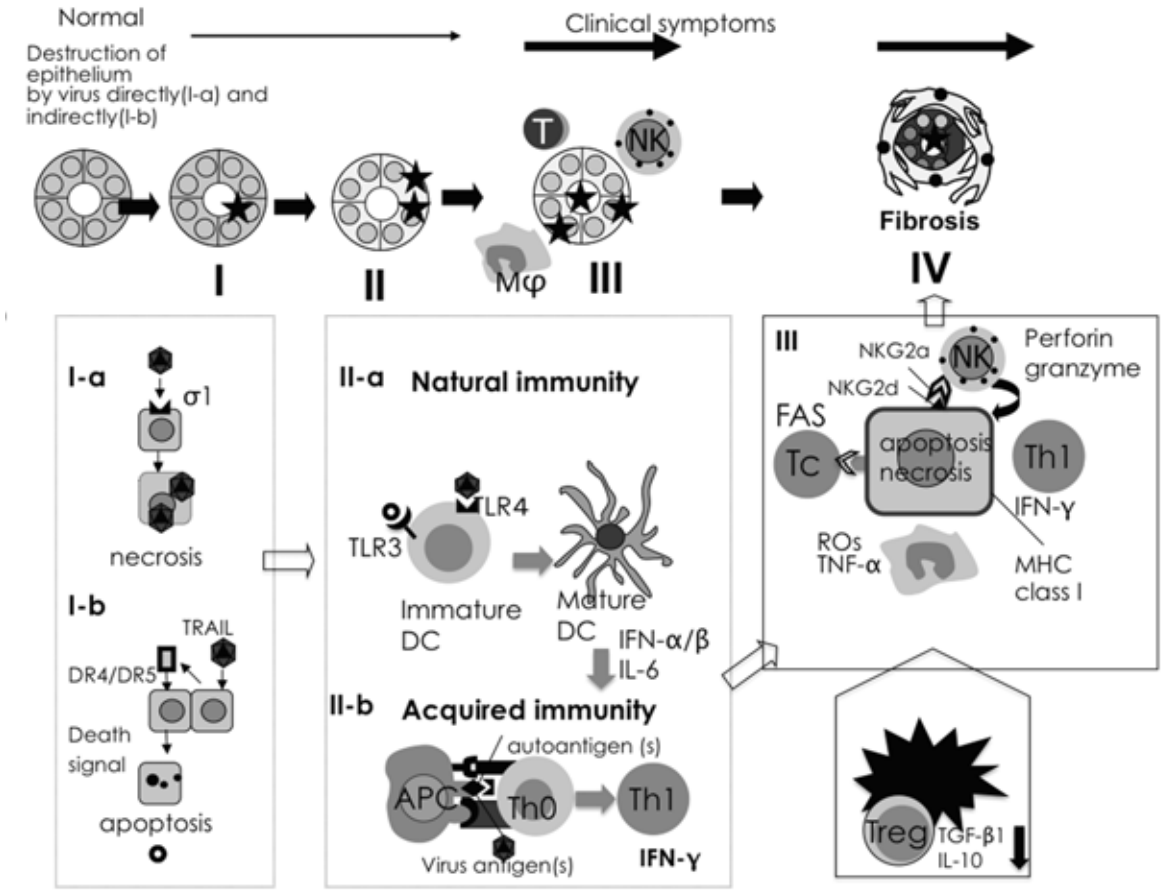

Fig. 2. Hypothetical mechanisms of immunological injury depending on our study using Reo-2-induced model for BA. Primary multiplication of virus in duct epithelium, leading to epithelium necrosis (I-a) and release duct epithelium autoantigens. At the same time, death signal via Reo-2 may induce apoptosis of epithelium (I-b). All of these may stimulate immature DCs (II-a) leading to the development of acquired immunity (II-b and III). Innate and acquired immunities eliminate Reo-2 from liver. IFN-α plays a role not only in elimination of virus but also may induce humoral and cell-mediated immunities against duct epithelial cells including enhanced expression of MHC-class I on duct epithelium under the influence of quantitative and qualitative impairments of Tregs. NK cell-mediated injury shown in rotavirus models seems likely in this process. Although the mechanisms of the development of sclerosing cholangitis by myofibroblasts besides fibroblasts are not known, continuous stimulation of environmental factors e.g. endotoxin such as LPS from intestines and the production of cytokines such as TGF-β being responsible for fibrogenesis may induce sclerosing BA (IV).

might be an essential precipitator or modulator once the required predisposing environmental events have occurred.

The concept of pathogenesis in other autoimmune diseases such as virus-triggered autoimmune type 1 diabetes (T1D) is suggestive to understand the pathogenesis of BA. According to multiple-hit concept proposed by Seline *et al.* [13], memory T cells are activated more efficiently than naive cells upon antigenic stimulation, and it has been shown that repeated infections using different viruses can lead to activation of memory T cells of unrelated specificity. They have indicated cytotoxic T (Tc) cell response to Pichinde virus (PV) and Vaccinia virus (VV) in C57BL/6 mice previously immunized with lymphocytic choriomeningitis virus (LCMV) including the reactivation of memory Tc cell specific to LCMV and that at least a part of this reactivation of memory T cells in LCMV-immune mice may be related to cross-reactivity at the clonal level [14]. Thus, it is possible that cumulative heterologous viral infections over a lifetime may lead to the generation of a memory pool of unrelated non-autoreactive cells as well as autoreactive cells in the absence of effective autoimmunity. In addition, a number of infections by viruses sharing epitopes with a self-antigen would lead to the selective accumulation of autoreactive T cells in this situation. Thus, encountering multiple related or unrelated infections during lifetime could lead to autoimmunity in genetically diabetes-prone individuals. In such a context, it is possible that one single viral event encountered before the onset of T1D, possibly unrelated to previous infectious events and not necessarily cross-reactive with self-antigens, can have the ability to trigger the final cascade leading to complete β-cell destruction and T1D when sufficient damage is inflicted to β-cells [14]. If this is the case, cumulative effects of pathogens such as bacteria and fungi may be involved together with virus.

Injury to self-tolerance by viruses

If BA is virus-triggered disease, induction of injury to self-tolerance by viruses will be required. This point is also mentioned again in the next section.

Hayashi *et al.* [15] reported the virus induced-suppression of regulatory T cells (Tregs), which maintain peripheral self-tolerance as follows. The frequency of $Foxp3^+$ cells in $CD4^+$ lymphocytes (Tregs), tend to be higher in Human T-lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis patients than in asymptomatic HTLV-I carriers and healthy control. The expression level of Foxp3 was found to be lower in HAM/TSP patients than in healthy control and was inversely correlated with the CMV-specific CTL frequency. A percentage of $Foxp3^+$ cells showed a positive correlation with the HTLV-I proviral load. These results suggest that a decrease in the Foxp3 expression may contribute to high immune response to CMV and that the $Foxp3^+$ Tregs may play a role in the immune surveillance of HTLV-I. This idea is very attractive and will give the break through to understand the pathogenesis of BA.

Innate and acquired immunities in destruction of bile ducts

There are two major immunities such as innate and acquired immunity, both of which contribute to the development of autoimmune diseases. However, the information of innate immunity in pathogenesis is totally lacking at present in BA. In autoimmune diseases such as T1D and SLE, innate immunity may play a role [16] in which interaction between virus components such as Toll-like receptor ligands (TLRs) and TLR on immature dendritic cells (DCs) may occur (Fig. 3). The presence of TGF-β and IL-6 may convert Th0 cells into Th17 cells which are responsible for the development of autoimmune diseases [16], although it is not proven in BA.

Shivakumar *et al.* [7] demonstrated, using a model of rotavirus-induced biliary atresia in newborn mice, that NK cells populated murine livers and were the most abundant cells in extrahepatic bile ducts at the time of obstruction, and that rotavirus-primed hepatic $NKG2a^+$ NK cells lysed $NKG2d^+$ cholangiocytes, which is an activated receptor ligand for NKG2a on NK cells. Depletion of NK cells and blockade of NKG2d, which is a suppressing receptor of NK cells and receptor for the major histocompatibility complex (MHC) class I on NK cells, each prevented injury of the duct epithelium after rotavirus infection. They concluded

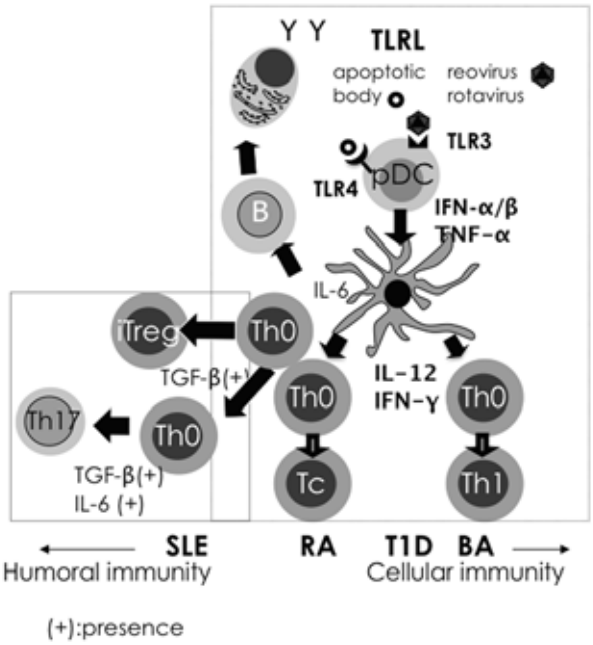

Fig. 3. Development of innate and acquired immunities in BA including several other autoimmune diseases.

NK cells as the key initiators of cholangiocyte injury via Nkg2d (Fig. 2).

Alternatively, cytokines from helper T (Th) 1 cell especially IFN-γ may contribute to virus-triggered injury of duct in BA. In addition, the components of reovirus act as toll-like receptor ligand (TLRL) on toll-like receptor (TLR) 3 on DCs. Also apoptotic bodies act as TLRL on TLR 9 on DCs, resulting in the production of IFN-γ. If that is the case, innate immunity may play a role, but information is limited in BA. There are two main DC differentiation pathways [17]: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) [18]. Resident mDCs that encounter bacteria at mucosal surfaces or at sites of tissue damage migrate to the lymph node via afferent lymphatics. Importantly, mDC express receptors, such as TLRs, nucleotide binding oligomerization domain (NOD) proteins, and lectins, to capture these pathogens. At the same time, IFN-α, which is produced by DCs, evoke strong Th1-polarized immune reaction [19]. During this process the absence of TGF-β and the presence of IL-6 may induce Th17 cells, which suppress the function of Tregs, resulting in enhancement of autoimmune reactions [19]. We reported the possibility of IFN-α that enhance autoimmune insulitis in mice infected with Reo-2 [20]. Also, Petersen *et al.* [21] reported that IFN-α treatment before rotavirus infection in mice prevented BA and IFN-α treatment 5 days after infection did not prevent BA, despite inhibition of viral growth.

Saxena *et al.* [22] found pDCs to be the most abundant DC population in the livers of newborn mice, and they observed pDCs in the livers of infants at the time of diagnosis. In the livers of newborn mice, conventional DCs spontaneously overexpressed the co-stimulatory molecule CD80 soon after birth, and pDCs produced IL-15 in response to a virus insult. Both subtypes of primed DCs were required for the proliferation of T lymphocytes and the activation of natural killer cells. Disruption of this cellular network by depletion of pDCs or blockade of IL-15 signaling in mice *in vivo* prevented epithelial injury, maintained anatomic continuity of the bile duct, and promoted long-term survival.

Mack [3] reviewed the evidence from both human and murine studies supporting a potential cholangiotropic viral infection as the initiator of bile duct injury in biliary atresia and the role of the adaptive immune response and autoimmunity in progression of disease. The theory of the pathogenesis of BA is that the bile duct damage is initiated by a virus infection followed by the release of altered “self” antigens that activate bile duct-specific autoreactive T cells, resulting in a chronic, inflammatory fibrosclerosing injury of the bile ducts as mentioned already in the earlier section. Mack [3] showed that $CD4^+$ Th1-mediated bile duct inflammation, which is consistent with the widely accepted hypothesis of BA, is responsible for the development of BA.

Impairment of self-tolerance

Underlying mechanisms of the development of autoimmune diseases are the destruction of self-tolerance. As already stated, there are two major cells such as Tregs and immature DCs which maintain peripheral self-tolerance [4, 23]. Diminished qualitative and quantitative changes of Tregs have been reported repeatedly in autoimmune diseases [19].

In order to address the possible role of autoimmunity in bile duct injury, liver T cell reactivity was

examined in older children with BA using murine CMV model as follows [4]. Brindley *et al.* [4] demonstrated the presence of virus infection associated with quantitative changes in Tregs. Liver T cells from BA and control patients were cultured with APCs in the presence of a variety of viral or control proteins. 56% of BA patients had significant increases in IFN-γ-producing liver T cells in response to CMV, compared with minimal BA responses to other viruses (e.g., rotavirus and reovirus) or the control group CMV response. In addition, a positive correlation between BA plasma CMV immunoglobulin M (IgM) and liver T-cell CMV reactivity was identified. Peripheral blood Tregs revealed significant deficits in Tregs frequencies in BA compared with controls, with marked deficits in those BA patients who were positive for CMV. Liver T-cell responses to CMV were identified in the majority of BA patients at diagnosis, suggesting perinatal CMV infection as a plausible initiator of bile duct damage. Deficiency of Tregs in BA implies decreased inhibition of inflammation and autoreactivity, potentially allowing for exaggerated bile duct injury. We have reported the evidence of reduced inducible Tregs (iTregs) in mesenteric lymph nodes and naturally occurring Tregs (nTregs) in thymus in Reo-2-infected mice [8], but the mechanisms of decrease in Tregs following this virus infection remain unclear.

Gene

It is well known that genetics play an important part in susceptibility to developing autoimmunity. The important relationship between genetics and autoimmune disease was reviewed by Kivity *et al.* [11] as follows. The role of genetics in the pathogenesis of autoimmune diseases is supported by their clustering in families and by a high concordance rate in twin studies (i.e. 24% in SLE, 25% in multiple sclerosis, 40% in T1D and 63% in primary biliary cirrhosis: PBC). The MHC genes and non-MHC–genes were found to be associated with autoimmune diseases and might serve as the ‘missing link’ between autoimmunity and various environmental factors. On the other hand, although the non-MHC autoimmune susceptible alleles are difficult to identify, since there is extensive genetic heterogeneity, they can be divided into tissue specific and immune regulatory genes. For example, CTLA-4 and PTPN22 are two non-MHC immune regulatory genes that are associated with T1D, autoimmune thyroid disease, SLE and RA. Tissue specific genes are NOD2 in Crohn’s disease. However, the relationship between genetics and cholangitis in BA is not defined. Chen *et al.* [24] reported altered expression of genes being responsible for hepatic morphogenesis and fibrogenesis. To elucidate factors involved in this process, they performed comprehensive genome-wide gene expression analysis using complementary DNA (cDNA) microarrays, and found that at least one third of BA markers were directly related to extracellular matrix (ECM) remodeling, hepatic morphogenesis, and cell adhesion molecules, but many of these molecules also play important roles in fibrogenesis. In addition, the collagens that are up-regulated in fibrogenesis were increased in expression in patients with BA. They confirmed MHC class II molecule up-regulation, but not the association between BA and HLA haplotypes. BA may result from an inherent defect in the epithelial-mesenchymal signaling pathways that would then lead to improper formation of the bile ducts at the porta hepatis during the first trimester. Although it is known that the up-regulation of collagen is due, in part, to overexpression of profibrogenic transforming growth factor (TGF)-β, they did not detect changes in TGF-β expression, which may have been a result of relatively low levels of messenger RNA expression of this cytokine.

Genome-wide association studies have identified BA susceptibility loci on several chromosomes [25, 26]. Leyva-Vega *et al.* [26] identified two unrelated BA patients with overlapping heterozygous deletions of 2q37.3. Patient 1 had a 1.76 Mb (280 SNP) heterozygous deletion containing 30 genes. Patient 2 had a 5.87 Mb (1346 SNP) heterozygous deletion containing 55 genes. These suggest that the overlapping 1.76 Mb deletion on chromosome 2q37.3 from 240936900 to 242692820 contains the critical region and that the genes within this region could be candidates for conferring susceptibility to BA. On the other hand, Garcia-Barceló *et al.* [25] identified a susceptibility locus for BA on 10q24.2. They showed that the likelihood of developing BA is

influenced by DNA variants in a region spanning 129 kb and encompassing the XPNPEP1 and ADD3 genes. These studies indicate that the identification of putative BA susceptibility loci not only opens new fields of investigation into the mechanisms underlying BA but may also provide new clues for the development of preventive and curative strategies [24]. Cui *et al.* [27] observed a statistically significant increase in deletions at 2q37.3 in patients with BA resulting in deletion of one copy of GPC1, which encodes glypican 1, a heparan sulfate proteoglycan that regulates Hedgehog signaling and inflammation. GPC1 appears to be a BA susceptibility gene. These findings also support a role for Hedgehog signaling in the pathogenesis of BA. However, the relation between these genetic abnormalities directly related to ECM remodeling and their involvement in autoimmune cholangitis in BA is unclear.

Regarding cholangitis, Zhao *et al.* [28] reported that the included genes were mainly involved in inflammation response and reconstruction of cellular matrix. The significant pathways associated with BA were primarily involved in autoimmune response, activation of T lymphocytes and its related cytokines. The RRAS, POMC, SLC26A6 and STX3 genes were important regulatory modules in the pathogenesis of BA. The expression of RRAS was negatively correlated with the elimination rate of jaundice and positively correlated with the occurrence rate of cholangitis. They pointed out that the RRAS gene is an important regulatory module in the pathogenesis of BA, which may serve as a novel prognostic marker for BA.

Sclerosing cholangitis

The mechanisms of progressing sclerosing cholangitis, which characterizes BA, are unsolved, compared to those of autoimmune-mediated destruction of bile ducts.

Nakanuma *et al.* [29] reviewed the etiopathogenesis of pediatric biliary diseases, such as BA. In an animal model of BA, an initial virus-induced, T-cell mediated autoimmune-mediated cholangiopathy has been reported as described in this review. In human BA, virus-induced apoptosis of BECs by a TNF-related apoptosis-inducing ligand followed by the progressive obliteration of bile ducts is also suggested, and epithelial mesenchymal transition of BECs induced by viral infection may be involved in the fibrotic process in sclerosing cholangitis. However, the role of viral infections in the affected tissues is controversial. Comprehensive and analytical studies of BA using human materials and animal models may be required.

GENERAL DISCUSSION

Developing further clarity on the points listed below may be required for the better understanding of the virus-triggered pathogenesis of BA.

1. As reviewed here a proposed trigger of this immune response is an initial viral infection, inducing biliary epithelial cells to become antigen-presenting cells and thus instigating immune-mediated destruction of the biliary tract (Fig. 3). Muraji *et al.* [30], however, questioned the triggering roles of viruses because putative viruses have never been confirmed. More recently, a novel hypothesis stating that maternal microchimerism may initiate a host immunologic response towards the bile duct epithelia has been proposed [31]. The fact that the pathology of BA develops during a period of biliary growth and remodeling suggests an involvement of developmental anomalies. Nakamura and Tanoue [31] proposed abnormal developmental theory for BA and indicated an association of the etiology of BA with some genetic factors such as laterality genes, epigenetic regulation and/or microRNA function.

2. Neonatal (within first three months of life) and extrahepatic bile duct involvement are primarily affected in BA. All mammals serve as hosts for reovirus infection, but disease is limited to very young people including mild disturbance of gastroenteritis [32]. One might expect that these may be determined by virus tropism and immunological immature status in childhood. The immaturity of infant immune systems may permit the establishment of persistent viral infections. Also, primary lesions in extra-hepatic bile ductal lesions suggest that the tropism of enterotropic viruses (e.g. reovirus and rotavirus) to bile duct epithelium and invasion of reovirus via duodenum may be one of the factors. Tan *et al.* [33] demonstrated the morphogenesis at the porta

hepatis level, because BA occurs during neonatal period. It was found that the primary biliary ductal plate undergoes a specific sequence of remodeling, resulting in the formation of large tubular bile ducts surrounded by thick mesenchyme, between 11 and 13 weeks post fertilisation. These developing ducts are in luminal continuity with the extrahepatic biliary tree throughout gestation. Contrary to long-held belief, no "solid phase" was observed in the development of the extrahepatic bile duct. Examination of the biliary remnants in biliary atresia showed that the porta hepatis is encased in fibrous tissue, and a variable pattern of obliteration of the common hepatic and common bile ducts was observed. Anti-cytokeratin immunostaining showed similarities between the abnormal ductules within the porta hepatis in biliary atresia, and the developing bile ducts in the first trimester. BA may be caused by failure of the remodelling process at the hepatic hilum, with persistence of fetal bile ducts poorly supported by mesenchyme. As bile flow increases perinatally, bile leakage from these abnormal ducts may trigger an intense inflammatory reaction, with subsequent obliteration of the biliary tree.

3. If autoimmune reaction induces BA, the underlying mechanisms may be due to impairment of self-tolerance at the cell level of Tregs and immature DC, which maintain self-tolerance. Focused study on these cells including antigen-specificity will be required.

4. BA is a multi-step disorder and the final sclerosing cholangitis leads to obstruction of bile ducts. For the development of sclerosing, continuous irritants or stimulation of autoreactive immune systems and induction of fibrogenesis will be required. One possibility is persistent virus infection. Also, cytokines may play a role in the development of fibrogenesis. The typical cytokines in fibrogenesis is TGF-β. However, TGF-β has multi-functions such as representative immunosuppressive effects other than induction of proliferation of fibrocytes. Further study is required to clarify the contradictory effects by TGF-β in the development of BA.

5. There are at least three immune-mediated duct injuries such as PBC, primary scrclosing cholangitis (PSC) and BA. What is the critical difference in terms of histology and immune-mediated pathogenesis other than the difference in primary lesions? Muraji *et al.* [34] demonstrated that microchimerism plays a crucial role in the pathogenesis of biliary atresia and they analyzed the localization of maternal microchimeric cells and their phenotypes as follows. Significantly larger numbers of maternal XX^+ cells were found in the portal area and sinusoids of patients with biliary atresia, in comparison with control patients. In phenotypic analyses of XX^+ cells, $CD8^+$ T cells, $CD45^+$ cells (memory T cells), and cytokeratin-positive cells were found, and the numbers and proportions among total $CD8^+$ T cells were significantly higher than those in control patients. Significantly more maternal chimeric $CD8^+$ T cells in the livers of patients with biliary atresia suggest that maternal immunologic insults represent the underlying pathogenesis in BA. The findings support the recently postulated mechanisms of alloautoimmune and/or autoalloimmune responses. They proposed that a new disease spectrum called maternofetal immune disease does not contradict the previously proposed etiologic considerations, such as viral infections or the ductal plate malformation theory. The viruses responsible may not necessarily be specific, because common hepatotropic viruses would be sufficient to activate the immunologic competency of the BECs to secrete inflammatory cytokines, which would make BECs more susceptible to T cell attack.

6. Increasing evidences indicate that most autoimmune diseases are Th1-polalized diseases, which suppress allergic diseases (most of them are Th2-polalized disease) by mutual inhibitory effects [35]. In the former case, it has been reported recently that Reo-1 prevented the development of allergy induced by peanut antigens in young mice [36]. Moreover, coexistence of both diseases is reported [35]. However, the relationship between allergic diseases and BA induced by viruses remain largely unknown.

7. The presence of human papillomavirus DNA in human liver with BA [37] or the detection of retrovirus proteins from human patients with BA [38] also suggest the etiologic agents of the disease. Thus, further study is required to clarify the etiologic roles of the disease.

8. Some viruses and their triggering roles in the pathogenesis of BA were introduced in this review. However, these viruses are suspected to be etiologic agents in T1D [39]. For example, (1) the appearance of diabetes-associated autoantibodies (GAD and IA-2) was associated with a significant rise in rotavirus antibodies, indicating that rotavirus infections may induce ß-cell autoimmunity in genetically susceptible infants. Molecular homology between the VP7 protein of rotavirus and T-cell epitopes in the IA-2 molecule and GAD65 molecule in humans has been reported. (2) CMV infection in humans can be congenital and also be transmitted perinatally or postnatally through close contact or breast milk. Infants with congenital CMV infection and a woman with CMV infection who both developed T1D, and the latter case developed T1D after extensive pancreatitis. In addition, (3) we have reported that Reo-2-triggered T1D in mice [31], although it is not proven in human patients with T1D. Actually, both rotavirus [40] and Reo-2 induce T1D in mice model [35]. We have no answer at present for such difference in terms of different autoimmune diseases induced by the same virus. However, if this is the case, BA is not an organ-specific disease.

CONCLUSIONS

The roles of viruses in the pathogenesis of BA are still undetermined. This review focuses on the triggering roles of viruses including participation of viruses in the development of BA. In addition, viruses could induce impairment of self-tolerance by several different ways. Taking these into consideration, viruses may be one of the important factors in the pathogenesis of BA.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

REFERENCES

1. Sokol, R. J. and Mack, C. 2001, Semin. Liv. Dis., 21, 517.
2. Landing, B. H. 1974, Prog. Pediattr. Surg., 6, 113.
3. Mack, C. L. 2007, Semin. Liver Dis., 27, 233.
4. Brindley, S. M., Lanham, A. M., Karrer, F. M., Tucker, R. M., Fontenot, A. P. and Mack, C. L. 2012, Hepatology, 55, 1130.
5. Shivakumar, P., Campbell, K. M., Sabla, G. E., Miethke, A., Tiao, G., McNeal, M. M., Ward, R. L. and Bezerra, J. A. 2004, J. Clin. Invest., 114, 322.
6. Shivakumar, P., Sabla, G., Mohanty, S., McNeal, M., Ward, R., Stringer, K., Caldwell, C., Chougnet, C. and Bezerra, J. A. 2007, Gastroenterology, 133, 268.
7. Shivakumar, P., Sabla, G. E., Whitington, P., Chougnet, C. A. and Bezerra, J. A. 2009, J. Clin. Invest., 119, 2281.
8. Nakashima, T., Hayashi, T., Tomoeda, S., Yoshino, M. and Mizuno, T. 2013, doi: 10.1038/pr.2013.170 (Epub ahead of print).
9. Lu, B. R., Brindley, S. M., Tucker, R. M., Lambert, C. L. and Mack, C. L. 2010, Gastroenterology, 139, 1753.
10. Feng, J., Yang, J., Zheng, S., Qiu, Y. and Chai, C. 2011, PLoS One, 6, e23655.
11. Kivity, S., Agmon-Levin, N., Blank, M. and Shoenfeld, Y. 2009, Trends Immunol., 30, 409.
12. von Herrath, M. G., Fujinami, R. S. and Whitton, J. L. 2003, Nat. Rev. Microbiol., 1, 151.
13. Seline, L. K., Nahill, S. R. and Welsh, R. M. 1994. J. Exp. Med., 179, 1933.
14. Nahill, S. R. and Welsh, R. M. 1993, J. Exp. Med., 177, 317.
15. Hayashi, D., Kubota, R., Takenouchi, N., Tanaka, Y., Hirano, R., Takashima, H., Osame, M., Izumo, S. and Arimura, K. 2008, J. Neuroimmunol., 200, 115.
16. Hayashi, T. 2010, J. Biomed. Biotech., 2010, 19.
17. Christen, U. and von Herrath, M. G. 2004, Mol. Immunol., 40, 1113.
18. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B. and Palucka, K. 2000, Annu. Rev. Immunol., 18, 767.
19. Liu, Y.-J. 2005, Ann. Rev. Immunol., 23, 275.
20. Nakashima, T., Hayashi, T., Yamamoto, Y. and Mizuno, T. 2012, Scand. J. Immunol., 76, 378.
21. Petersen, C., Brunus, E., Kuske, M. and von Wussow, P. 1997, Peddiatr. Res., 42, 623.
22. Saxena, V., Shivakumar, P., Sabla, G., Mourya, R., Chougnet, C. and Bezerra, J. A. 2011, Sci. Transl. Med., 28, 102.

23. Feldman, A. G. and Mack, C. L. 2012, Semin. Pediatr. Surg., 3, 192.
24. Chen, L., Goryachev, A., Sun, J., Kim, P., Zhang, H., Phillips, M. J., Macgregor, P., Lebel, S., Edwards, A. M., Cao, Q. and Furuya, K. N. 2003, Hepatology., 38, 567.
25. Garcia-Barceló, M. M., Yeung, M. Y., Miao, X. P., Tang, C. S., Cheng, G., So, M. T., Ngan, E. S., Lui, V. C., Chen, Y., Liu, X. L., Hui, K. J., Li, L., Guo, W. H., Sun, X. B., Tou, J. F., Chan, K. W., Wu, X. Z., Song, Y. Q., Chan, D., Cheung, K., Chung, P. H., Wong, K. K., Sham, P. C., Cherny, S. S. and Tam, P. K. 2010, Hum. Mol. Genet., 19, 2917.
26. Leyva-Vega, M., Gerfen, J., Thiel, B. D., Jurkiewicz, D., Rand, E. B., Pawlowska, J., Kaminska, D., Russo, P., Gai, X., Krantz, I. D., Kamath, B. M., Hakonarson, H., Haber, B. A. and Spinner N. B. 2010, Am. J. Med. Genet., 152A, 886.
27. Cui, S., Leyva-Vega, M., Tsai, E. A., EauClaire, S. F., Glessner, J. T., Hakonarson, H., Devoto, M., Haber, B. A., Spinner, N. B. and Matthews, R. P. 2013, Gastroenterology, 144, 1107.
28. Zhao, R., Li, H., Shen, C. and Zheng, S. 2011, World J. Gastroenterol., 17, 796.
29. Nakanuma, Y., Harada, K., Sato, Y. and Ikeda, H. 2010, Histol Histopathol., 25, 223.
30. Muraji, T., Suskind, D. L. and Irie, N. 2009, Expert Rev. Gastroenterol. Hepatol., 3, 599.
31. Nakamura, K. and Tanoue, A. 2013, J. Hepatobiliary Pancreat. Sci., 20, 459.
32. Onodera, T. and Hayashi, T. 2012, Reovirus, H. Hyoty, K. Taylor, A. Toniolo and A. Zuckerman (Eds.), Springer, New York.
33. Tan, C. E., Driver, M., Howard, E. R. and Moscoso, G. J. 1994, J. Pediatr. Surg., 29, 808.
34. Muraji, T., Hosaka, N., Irie, N., Yoshida, M., Imai, Y., Tanaka, K., Takada, Y., Sakamoto, S., Haga, H. and Ikehara, S. 2008, Pedatrics, 121, 517.
35. Hayashi, T. 2010, Chapter 1, In Handbook type 1 diabetes mellitus, L. Aucoin and T. Prideux (Eds.), Nova Science Publishers Inc., New York.
36. Fecek, R. J., Marcondes Rezende, M., Busch, R., Hassing, I., Pieters, R. and Cuff, C. F. 2010, Immunobiology, 215, 941.
37. Drut, R., Gomez, M. A., Drut, R. M., Cueto, R. E. and Lojo, M. 1998, Acta Gastroenterol. Latinoam., 28, 27.
38. Mason, A. L., Xu, L., Guo, L., Munoz, S., Jaspan, J. B., Bryer-Ash, M., Cao, Y., Sander, D. M., Shoenfeld, Y., Ahmed, A., Van de Water, J., Gershwin, M. E. and Garry, R. F. 1998, Lancet, 351, 1620.
39. Onodera, T., Taniguchi, T., Yoshihara, K., Shimizu, S., Satoh, M. and Hayashi, T. 1990, Diabetologia, 33, 192.
40. Honeyman, M. C., Coulson, B. S., Stone, N. L., Gellert, S. A., Goldwater, P. N., Steele, C. E., Couper, J. J., Tait, B. D., Colman, P. G. and Harrison, L. C. 2000, Diabetes, 49, 1319.