Vanishing Bile Duct Syndromes: Considerations of the Immunobiology of Autoimmune Biliary Diseases

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Bile is a complex aqueous mixture containing organic and inorganic compounds in true solution, as well as suspended micelles of organic compounds. The major organic compounds are bile acids, bile pigments, cholesterol and phospholipids. The major inorganic solutes are sodium, chloride and bicarbonate. A variety of enzymes and other proteins are also present, albumin being the most common protein followed by immunoglobulins. Thus, the concentration ranges of the major biliary proteins have been determined to be from 155 to 1,485 μg/ml for albumin, 32–480 μg/ml for IgG, 11.8–66.7 μg/ml for monomeric IgA, 25.3–146 μg/ml for polymeric IgA, 2.2–60 μg/ml for IgM, 6–66.4 μg/ml for orosomucoid, 11.4–160 μg/ml for transferrin, and 2.7–100 μg/ml for alpha-2-macroglobulin [1].

Bile provides an excretory pathway for numerous metabolites, such as bile acids and bile salts (degradation products of cholesterol), bile pigments (degradation products of heme), cholesterol, heavy metals, and conjugates with excretory enzymes including drug metabolites. Bile acids and salts are necessary for the emulsification and subsequent absorption of fats from the small intestine. Bile is formed initially by hepatocytes via the net movement of water and solutes into the bile canaliculi. Bile canaliculi are spaces, one micron in diameter, between adjacent hepatocytes bound by the canalicular (apical) domains of the hepatocyte plasma membranes, and segregated from the intercellular space and sinusoidal blood (basolateral surface) by junctional complexes. Neighboring canaliculi join together and empty their contents into small terminal ductules (canals of Hering). The bile is then conveyed to large ductules, intralobular ducts, interlobular ducts, and eventually to the extrahepatic bile ducts through which it flows to the gallbladder and the duodenum. As the nascent bile travels through the biliary tree, it is modified by the biliary epithelium in the intrahepatic ductules and ducts as well as in the extrahepatic ducts and gallbladder.

Protein Content of Bile

Substantial quantities of proteins are found in mammalian bile. Many of the biliary proteins are immunologically similar to serum proteins, suggesting that specific proteins may be transported from serum into bile. Such serum-derived proteins might enter bile either by diffusion across the tight junction between hepatocytes and/or biliary epithelial cells or via a trans-hepatocellular vesicular route [2], using either fluid-phase endocytosis or receptor-mediated endocytosis [3]. Most proteins enter by passive fluid-phase endocytosis and include albumin, immunoglobulins, alpha-2-macroglobulin and alpha-1-acid glycoprotein [4]. Entry of detectable amounts of protein into the bile by this route is dependent upon the serum concentration of the respective protein [2]. On the other hand, receptor-mediated endocytosis is responsible for the biliary secretion of an IgA receptor complex, as well as small amounts of epidermal growth factor, transferrin, hemopexin and asialoglycoprotein [5].

Although most proteins present in bile are derived from plasma, data indicate that other proteins are secreted into bile from the hepatocytes without entering into the plasma [4]. For instance, the hepatic Iysosomal proteins excreted into bile appear to be a major pathway for eliminating the contents of hepatic lysosomes [6]. In this regard, fusion of lysosomes with the canalicular membrane has not been proven, although there is a change in the number of pericanalicular lysosomes following exposure to agents that affect lysosomal discharge into bile [6]. Furthermore, several proteins normally found in the hepatocyte canalicular membrane are released into bile at low rates [3]. Interestingly, proteins may enter bile by more than one pathway. For example, albumin can enter bile from serum via the paracellular/transcellular route, using passive fluid-phase endocytosis, or it can be secreted from the hepatocyte directly into the bile without entering the serum. In fact, it has been proposed that the external hemileaflet of the canalicular plasma membrane sloughs off into bile. It appears that detergent hydrophobic bile salts promote canalicular membrane secretion [7], implicating a detergent interaction between secreted bile salts and the external aspect of the canalicular plasma membrane. This bile salt-induced exocytosis of the external hemileaflet of the canalicular plasma membrane appears to be responsible for the biliary secretion of phosphatidylcholine. Crawford [3] speculated that the exocytosis of the external hemileaflet may drag canalicular membrane proteins into nascent vesicles prior to their release into bile.

Biliary Immunoglobulins

Bile has been intensively studied as a means of delivering immunoglobulin into the intestine. Much of the data on the sources of immunoglobin in bile have come from experiments in rats. The presence of Iymphoid cells that

contain immunoglobulin in the livers of human and experimental animals is well accepted; however there is some controversy over the relative contribution of intrahepatic lymphocytes to antibodies in bile. It is presently thought that biliary immunoglobulin is derived both from blood and from local production with subsequent transportation.

It has been known for 30 years that secretory IgA is the predominant class of immunoglobulin present on mucous membranes that are exposed to the external environment. sIgA[1] is the major immunoglobulin in rat bile, and ligation of the rat bile duct leads to appearance of slgA in serum [8]. Moreover, cannulation of the rat bile duct results in a tenfold reduction of IgA in the intestinal fluid [8], while the injection of polymeric IgA into the systemic circulation or into the portal vein of the isolated liver leads to its transport into bile [9].

The source of multimeric IgA in human bile is controversial. Nagura et al. [10], who found sparse IgA-containing plasma cells in portal triads and only modest numbers in the mucosa of extrahepatic bile ducts, concluded that local synthesis was an unlikely source for the large quantities of IgA in bile. These investigators considered a vascular or a lymphatic source, although thoracic duct lymph had been found to have mainly monomeric IgA [11]. Conversely, Delacroix et al. [1] demonstrated a selective transport of polymeric IgA in bile from both blood and local origin. They suggested that serum plgA[2] in blood contributes to about half of this selective excretion in bile, while the other half of plgA in bile is likely to come from a local contribution of IgA plasmacytes present in the portal tract and biliary submucosa. Indeed, other investigators have postulated that local synthesis by plasma cells in the liver and biliary tract accounts for about 88% of the monomeric IgA, 50% of the pIgA, and an undetermined portion of the IgM [1] found in human bile. It has also been proposed that the remaining IgA is synthesized in the lamina propria of the gut-associated lymphoid tissue and reaches the gut lumen via the lymph to the circulation and thence into the bile [l]. Furthermore, it has been suggested that IgA enters the venous drainage of the gut and reaches the liver directly via the portal vein without entering the systemic circulation [Figure 1].

The major pathway for the transfer of plgA into bile is through a receptor-binding interaction. Studies on bile transport in rats have shown that plgA is transferred by an extracellular receptor known as the secretory component, which is present in rat hepatocyte and binds IgA [12]. The synthesis of the SC[3] by cultured rat hepatocytes and its binding of plgA has also been demonstrated [13]. Other studies have confirmed that, in the rat, a substantial source of the biliary IgA comes from the hepatocyte by receptor-mediated endocytosis en route to the intestine. It appears that this mechanism delivers over 90% of endocytosed IgA as a polymeric IgA receptor complex to the bile [14].

Importantly, the mechanisms of transport of IgA from serum to bile are greatly influenced by the species under study. Immunohistochemical studies have revealed species differences in the localization of SC. Thus, SC was localized in the hepatocytes in the rat, rabbit and mouse, whereas it was predominantly localized in the BECs[4] of human, dog and guinea pig [10,15]. In humans, IgA is the most important protein transported from plasma to bile by transcytosis. It has been reported that in humans, the epithelial cells of biliary ductules are involved in the synthesis of the SC and the transport of slgA into the bile [5]. It is believed that IgA passes through the cells by first binding to the membrane SC in the basolateral side of the cell [15]. Using isolated and cultured cells, Sakisaka et al. [16] demonstrated that labeled plgA2 was transported transcellularly via the tubulovesicular pathways and secreted into the bile canalicular space in cultured rat hepatocytes, while it was transported via the tubulovesicular pathway and secreted into the luminal spaces in cultured guinea pig BECs.

The study of the movement of IgA across the rat hepatocyte has shown that the IgA–mSC complex is internalized within endocytic vesicles [17]. Following endocytosis, the IgA–mSC[5] complexes are localized in a vesicular–tubular network, where presumably the sorting of IgA from other endocytosed ligands occurs. Once sorted, the IgA–mSC-containing vesicles migrate across the cell, avoiding interaction with lysosomes, and fuse with the bile canalicular membrane [17]. The IgA delivery pathway ends with the proteolytic solubilization of the IgA–mSC complex at the bile canalicular pole of the hepatocyte. It is here that the mSC is proteolytically processed to yield two proteins: a soluble form of SC that is released into bile still as a complex with IgA, and a transmembrane anchoring fragment that contains the cytoplasmic tail of mSC and remains with the membrane. Studies on cultured BECs have shown that they possess a tubulovesicular transport system similar to that identified in isolated rat hepatocytes [18].

It has been shown that the transport of plgA into bile in several species, particularly the rat, depends on the interaction between mSC on hepatocytes and circulating plgA; however, biliary secretion of plgA in humans has not been totally elucidated and may in part involve hepatic receptors other than mSC. IgA1 (both monomeric and polymeric) has been shown to bind to human liver homogenates and Hep2 cells by the asialoglycoprotein receptor [19]. Schiff et al. [20] suggested that in the rat hepatocytes, which possess both the ASPGR[6] and the mSC, human IgA1 can be internalized via the ASPGR, and once inside the cell, can dissociate from the ASPGR due to low ph, bind to mSC, and be routed into the bile. The ASPGR's role in biliary secretion of human IgA is still under study. Human IgA1 contains a galactose terminal O-linked oligosaccharide in the hinge region of the molecule that may allow the IgA1 to interact with the ASGPR.

The concentration of biliary IgG is less than 8% the concentration of serum IgG [21]. Virtually all IgG appears to enter human bile by diffusion from blood. However, in a study on human bile after tetanus toxoid immunization the kinetics of the biliary IgG response did not follow that in

[1] sIgA = secretory immunoglobulin A
[2] pIgA = polymeric IgA
[3] SC = secretory component
[4] BECs = biliary epithelial cells
[5] mSC = membrane secretory component
[6] ASPGR = asialoglycoprotein receptor

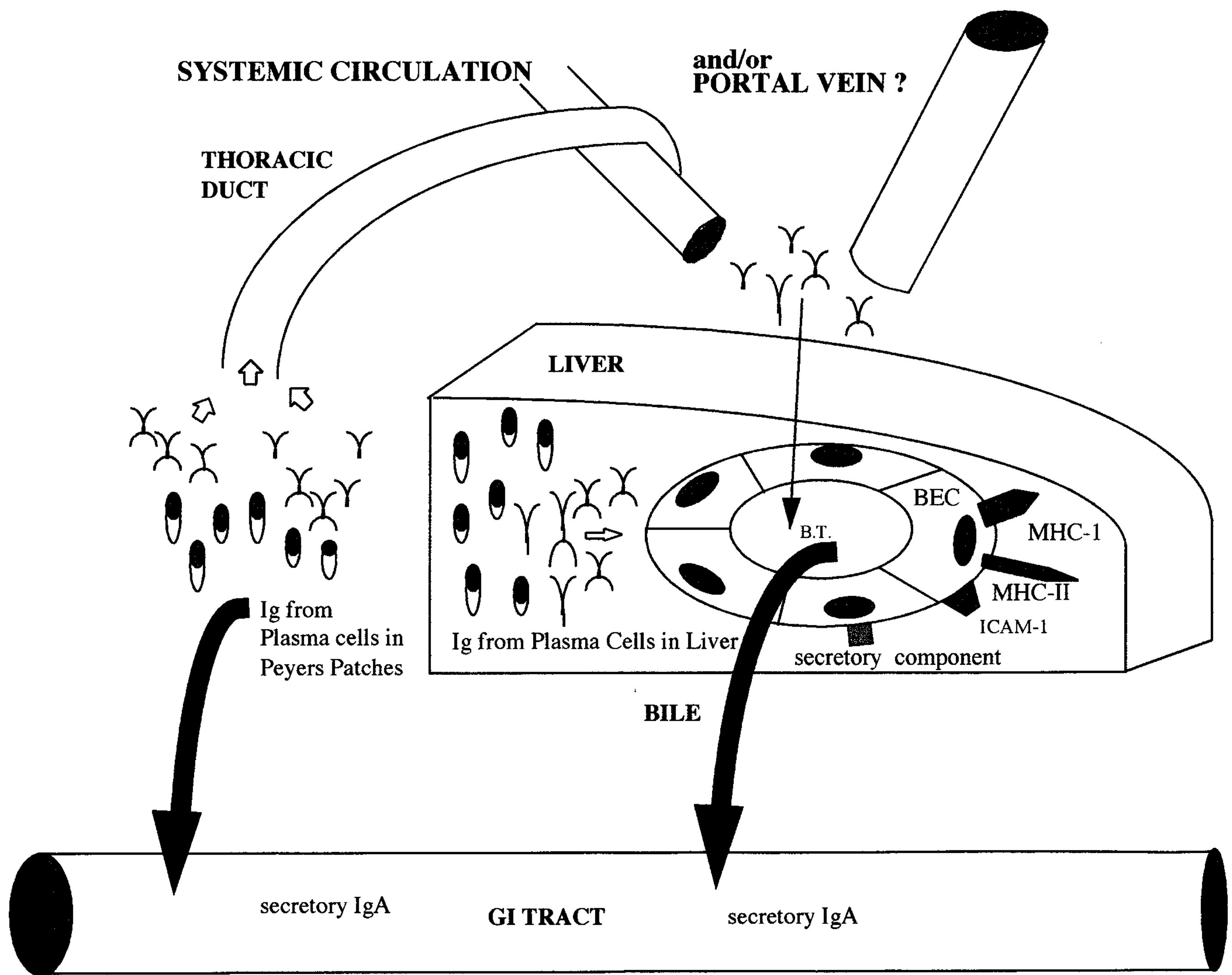

Figure 1. BECs upregulate MHC I, MHC II and ICAM-1. They also express secretory components, which aid in the transport of IgA into the bile. The presence of biliary monomeric and polymeric IgA might be due to the local synthesis of plasma cells in the liver and biliary tract. IgA might also be synthesized by plasma cells in the lamina propria of the gut-associated lymphoid tissue, reaching the bile through the thoracic duct and systemic circulation. IgA might also reach the liver directly via the portal vein without entering the systemic circulation. BT = biliary tract, GT = gastrointestinal tract.

serum, and comprised more antibody per total IgG than could have arrived by diffusion [22]. Thus, tetanus toxoid immunization of humans resulted in an excess of biliary IgG and IgA antibody, suggesting intrahepatic antibody production for export to the intestinal tract in humans. Indeed, Manning and co-workers [21] showed that after thoracic duct cannulation in rats the fall in serum IgG concentration was not comparable to that seen in bile. One explanation is that the serum level of IgG is still considerably higher after cannulation, and that the concentration gradient leads to passive exudation into the bile. Alternatively, the numerous IgG plasma cells found in liver could maintain the bile IgG concentration observed.

The origin of the trace amounts of IgM in bile is controversial. It may be a result of a leakage from plasma, although the transient increase of biliary IgM that occurs after immunization of rats could be attributed to the localization in liver of IgM-producing cells [23]. The latter is supported by studies of Jackson et al. [24], who proposed that antigen entering the bloodstream stimulates a population of cells in the spleen that emigrate to the liver, where the cells localize and secrete IgM into bile.

Presence of Natural and Antigen-Specific Immunoglobulin in Bile

Immunogenic stimulation by antigens, such as those induced by normal flora that are present in the local environment particularly within the intestinal tract, could account for the appearance and maintenance of the natural antibodies in bile. On the other hand, several investigators have reported the presence of various antigen-specific immunoglobulins in bile. One study reported that immunization in Peyer's patches of rats with horse spleen ferritin or *Escherichia coli* 06 carrying type 1 pili resulted in a specific IgA, IgG and IgM antibody response in bile [25]. Another study indicated that specific IgG and IgA was found in human bile during responses to parenteral tetanus vaccination [22]. Other studies have reported the presence of specific IgA in bile after duodenal inoculation of live *Giardia lamblia* in rats [26]; and that patients with clonorchiasis infestation have specific antibodies in bile to antigens of adult *Clonorchis sinensis*, with a significant correlation between the levels of IgG and IgA antibodies in bile and the intensity of infection [27].

In a recent study [28], primary biliary cirrhosis antimitochondrial antibodies were detected in the bile at a ratio of 1.55±0.95 (mean±SEM) to that found in sera. The specific antimitochondrial antibodies in bile always corresponded in specificity with those present in the serum. Furthermore the actual autoantigen, the E2 subunit of the pyruvate–dehydrogenase complex, was also detected in the bile of these patients.

Biological Significance of Immunoglobulins in Bile

The biological significance of the transport of plgA from the plasma into bile is not yet clearly defined, but there are at least three proposed functions. First, the presence of IgA in bile might be one mechanism for enhancing the immune protection of the biliary tract and intestine by preventing the attachment of injurious microorganisms or their toxins to mucus membranes. SIgA aggregates bacteria, inhibits their motility, and prevents their adherence to epithelial cells [29]. As mentioned above, natural IgA antibodies to intestinal bacteria are present in bile, and in numerous studies the inoculation of various antigens into the intestinal lumen or intestinal lymphoid tissues has resulted in the secretion of specific IgA antibodies into bile.

Second, the pathways of IgA through bile may constitute a mechanism for clearing harmful antigens from the circulation in the form of immune complexes with plgAs, thus reducing the likelihood of a systemic response [30]. This route for excreting locally formed IgA immune complexes would not only reduce their chance of reaching the circulation, but may possibly help prevent immune complex diseases. Because IgA induces a much lower inflammatory response than IgG or IgM, it would seem to be ideal for such a local excretory role. This could be especially important in the gastrointestinal tract, where local immune complexes might be present regularly and where it would be advantageous to have mechanisms that could prevent a state of chronic inflammation [31]. It is relevant here to note that IgA-deficient individuals are prone to develop autoimmune and immune complex diseases [32].

Trans-hepatic delivery of pIgA into bile can serve to eliminate antigens, such as haptenated proteins, complexed with anti-hapten IgA antibodies [33]. Indeed, the transport of intravenously injected free antigen into bile by circulating IgA antibody — either passively administered or actively induced — has been demonstrated [33,34]. In 1981, Peppard et al. [30] demonstrated that the excretion into bile of complexes formed by human polymeric monoclonal IgA and rabbit anti-IgA occurs via a mechanism involving the SC. It was later demonstrated that transport *in vivo* of an antigen from blood to bile occurred by endogenous polyclonal IgA antibodies during an immune response [34]. In 1983, Russell et al. [35] used a mouse model to demonstrate that a similar process of excreting immune complexes occurs with bacterial polysaccharides and corresponding monoclonal IgA antibodies. Based on sedimentation analysis, these researchers showed that the antigens transported into bile by IgA antibody were at least partially intact and appeared in the form of high molecular weight complexes. They suggested that degradation of the antigen is not necessary for transport, hence IgA-mediated hepatobiliary transport may constitute a means of eliminating undegradable antigens, as well as immunologically reactive antigens and their fragments [35]. However, partial dissociation of IgA antigen complexes has been reported by others [34]. Russell and co-workers went a step further, suggesting that the molecular weight determined the type of immune complexes that are eliminated through the bile. These investigators suggested that complexes smaller than 106 kDa are amenable to hepatobiliary transport, whereas larger ones may be cleared by the mononuclear phagocytic system. Only the former mechanism would eliminate an undegradable antigen. Later, Rifai and Mannik [36] reported that high molecular weight complexes were cleared from the circulation by the non-parenchymal cells of the liver but were not transported into bile. However, the molecular weight of these complexes was larger than the limit specified by Russell's team [35] for efficient hepatobiliary transport. It is still unclear whether incompletely digested antigens released from the phagocytes can be eliminated by IgA-mediated hepatobiliary transport.

Lamm et al. [31] suggest that when a molecule of IgA binds to the polymeric Ig receptor, a second molecule can bind directly or indirectly to the IgA and can be transported across the epithelial cells. This second molecule or molecules could be an antigen or an antigen bound to an IgG, for example. Under natural conditions, in which other classes of immunoglobulin are present in the mucosal lamina propria in addition to the predominant IgA, it is possible that IgG antibody may be in the same immune complex as IgA and that this mixed immune complex may be subject to secretion through epithelium.

A third function for the transport of IgA from plasma into bile has been proposed, which originates from the fact that IgA does not pass between the lining epithelial cells, whose apical margins are connected by tight junctions. Instead, as mentioned above, IgA passes through the epithelial cells by transcytosis. This transcellular route of secretion leads to the possibility that IgA antibodies can encounter an intracellular microbial pathogen or fragments thereof, infecting the same epithelial cells through which the IgA is passing [31]. Mazanec et al. [37] studied this possibility in a system composed of a polarized epithelial cell monolayer located in a two-chambered vessel, with the monolayer situated between apical and basal compartments that could be separately manipulated. Sendai or influenza virus could be introduced into the apical compartment to be endocytosed from below, possibly affording an opportunity for antibody and virus antigen to meet intracellularly. The epithelial cells in this study were derived from canine renal tubule cells that had been transfected so as to express the rabbit polymeric immunoglobulin receptor and, therefore, were capable of transcytosing IgA. Experiments using two-color immunofluorescence showed that IgA antibody to viral envelope protein introduced from below co-localized with viral protein within the epithelial cells that had been infected from above [37,38]. Thus, some specific IgA was being transported across the cell and had access to viral protein.

Lamm et al. [31] studied the neutralization of virus in epithelial cells *in vivo* by intravenous injection of IgA monoclonal antibodies, which recognized a specific viral protein after mice had been exposed to the specific virus

via the respiratory tract. Mice given the IgA antibody had lower virus titers in nasal and bronchoalveolar washes compared to mice that had received IgG antibody. These investigators concluded that some of the intravenously administered IgA antibodies entered the epithelial cells in the respiratory tract and interfered with virus replication. Thus, mucosal defense mediated by IgA antibodies may well protect against intracellular pathogens in the biliary epithelium, a role also assigned to T cell-mediated immune mechanisms.

Bile during Disease

Although bacteria are not present in the normal human biliary tract, they are demonstrable in the bile of patients with bile duct inflammation [39], in some 65% of patients with choledocholiathiasis [40], and even more frequently (90%) in selected groups of patients with inflammation of the bile duct wall [41]. The origin of such bacteria has not been definitely established, but it may occur by ascending infection since the species identified are those commonly present in the normal duodenum and jejunum. Another explanation is that bacteria are derived from an infected gallbladder. Whatever the case, further studies are needed.

In the case of biliary immunoglobulin in disease states, patients suffering from cholestasis due to biliary obstruction have an impaired excretion of IgA by the liver and elevated levels of IgA in the serum. Oshio et al. [42] measured the serum levels of IgA immune complexes and the endotoxin levels in 33 patients with biliary obstruction and cholangitis. The endotoxin levels correlated with the amount of IgA immune complexes in blood, suggesting that impaired IgA transport might contribute to the entry of endotoxin into the circulation, which resulted in septicemia and shock [42]. When biliary obstruction was relieved, serum slgA levels returned to normal. In a study of patients with cholelithiasis [43], bile slgA levels of patients with brown pigment stones were significantly lower than in those in a control group. Moreover, the bile slgA values in the patients with cholelithiasis and active cholangitis were lower than those in patients without infection. This strengthens the suggestion that slgA, through its antibacterial adherence effect, is important for prevention of microbial colonization of the biliary epithelium.

Bile Duct Epithelium and Bile

The bile duct is surrounded by epithelium, consisting of heterogeneous cell types capable of sustaining a variety of transport functions that contribute to the final bile composition. Intrahepatic bile duct epithelial cells comprise only 3–5% of the total population of liver cells, yet produce as much as 40% of the daily output of bile, depending on the species. At the same time they modify the organic and inorganic constituents of bile through many different reabsorptive mechanisms [44]. However, progress in understanding the immunobiology of BECs has been limited by the technical problem of isolating this lineage from their more numerous neighbors. Advances were made when it became possible to isolate a larger number of cells from bile ducts of animals by using biliary obstruction with differential gradient centrifugation and centrifugal elutriation techniques [45]. A major problem with such a procedure is the origin of the cells, since the bile duct epithelium is anatomically highly heterogeneous. It begins with the small canals of Hering in the periportal region of the liver where the cells are small, and increases progressively in diameter on transition to interlobular ducts, septal ducts, segmental ducts, and finally into main branches of the extrahepatic bile duct and common bile duct system. To date, it has not been possible to cleanly isolate BECs from any specific area of the biliary tract.

Biliary Epithelium and its Interactions with the Immune System

There are several mechanisms by which BECs might contribute to the regulation of the immune inflammatory responses. These cells promote the recruitment and activation of circulating leukocytes by secreting chemokines and cytokines. Recent studies have investigated whether human BECs can produce interleukin-8 and monocyte chemotactic protein-1 and thereby play a role in promoting leukocyte recruitment to portal tracts — either as part of a protective response against ascending biliary infection or as participants in the pathological inflammation of bile ducts that characterizes allograft rejection. Morland et al. [46] have shown that human BECs do express and secrete functional chemokines; they have the ability, when stimulated by pro-inflammatory cytokines such as IL-1 or tumor necrosis factor-α, to secrete two chemotactic cytokines, IL-8 and monocyte chemotactic protein-1, that facilitate recruitment of neutrophils and lymphocytes into tissue [46]. These findings demonstrate that BECs are indeed active participants in the generation of portal inflammation. The ability of BECs to express and secrete chemokines is probably important for the processes of immune defense of the biliary tract. The biliary epithelium is in contact with potentially pathogenic microorganisms present in the bile or ascending from the intestine via the biliary tract; and, as mentioned, neutrophils are recruited to the bile ducts and infiltrate the biliary epithelium in cases of ascending cholangitis or biliary obstruction. BEC-derived chemokines, secreted in response to cytokines such as TNF-α[7] that are released early in the course of bacterial infection, might be crucial in the initial recruitment and activation of leukocytes to oppose invading pathogens.

BECs could intensify and localize the immune response by expressing select cell adhesion molecules. Enhanced expression of such select cell adhesion molecules in areas of hepatobiliary damage has recently been identified in several liver diseases. BEC expression of intercellular adhesion molecule-1 has been demonstrated both *in vivo* and *in vitro* in response to inflammatory stimuli [47]. The expression of ICAM-1[8] on BECs facilitates strong adhesion between BECs and cytotoxic T lymphocytes that express the cognate ligand for ICAM-1, termed the leukocyte function-associated antigen-l. Also, BECs might act as antigen-presenting cells, actively stimulating lymphocytes to respond to specific portal antigens. The potential ability of BECs to present antigen to T cells is still a controversial issue. By inducing with interferon-γ, Ayres confirmed that

[7] TNF-α = tumor necrosis factor-α

[8] ICAM-1 = intercellular cell adhesion molecule-1

cultured BECs can express major histocompatibility complex class II [48]; however, in order to act as a competent antigen-presenting cell for inducing primary immune responses, co-stimulatory molecules such as CD40, CD80 and CD86 must be expressed on their surface. Leon and his team [49] were unable to induce the expression of B7-1 (CD80) or B7-2 (CD86) *in vitro* by activation with high doses of IFNγ, TNF-α or phorbol myristate acetate [49]. Thus, conflicting results exist on the actual expression of co-stimulatory molecules on human BECs.

BECs as Targets in "Vanishing Bile Duct Diseases"

There is growing evidence that BECs actively participate in the generation of portal inflammation in liver disease. Moreover, BECs could act as targets for cytotoxic T lymphocytes by presenting antigens and expressing adhesion molecules that render them susceptible to cytolytic mechanisms [50]. Given the likely ability of BECs to express MHC[9] antigens (class I and II) and adhesion molecules such as ICAM-1 that are required for the adhesion of cytotoxic T lymphocytes, it is not surprising that BECs can act as targets for cytolytic T cells [51]. BECs upregulate ICAM-1 both *in vivo* and *in vitro* in response to inflammatory stimuli [48,52]. Adams [51] suggests that the presence of ICAM-1 facilitates strong adhesion to occur between BECs and cytotoxic T lymphocytes. The presence of antigen in the form of peptide in association with MHC could thus be recognized by cognate T cell receptors on CTLs[10], leading to CTL activation, effector function, and apoptosis of the biliary cell.

Recently, efforts have been directed to elucidate the involvement of biliary epithelium as a target for toxic and immunologic injury, particularly in relation to the development of the interesting group of "Vanishing Bile duct diseases." These include primary biliary cirrhosis, primary sclerosing cholangitis, graft-versus-host disease, and allograft rejection in which progressive rejection of a liver allograft includes the disappearance of interlobular bile ducts [53,54]. In PBC[11], ICAM-1 is strongly expressed on BECs, involving about half of the damaged bile ducts, whereas vascular cell adhesion molecule-I is expressed on a few of the damaged bile ducts [55]. In PBC, many portal tract lymphocytes express leukocyte function-associated antigen-1, VLA-4, ICAM-1 and VCAM-1[12], suggesting ICAM-1/LFA-1[13] and VCAM-1/VLA-4 interactions between damaged bile ducts and lymphocytes. Aberrant expression of MHC class II antigens by BECs early in the course of disease in PBC has been suggested as a means by which the bile duct epithelial cells may serve as antigen-presenting cells for bile duct autoantigens. The overexpression of MHC class I and II, however, might be a nonspecific consequence of the portal inflammatory environment [56].

As in PBC, aberrant expression of MHC class II by BECs occurs in PSC[14], chronic GVHD[15] and liver allograft rejection — diseases characterized by lymphocyte-mediated bile duct destruction. Intrahepatic BECs express co-stimulatory molecules such as CD40, CD80 and CD86 in the later, but not in the earlier stages of PBC. Studies in patients with allograft rejection and PSC, however, have shown negative results for the expression of CD80 [49,55]. Interestingly, Hu et al. [50] used murine BECs obtained by bile duct ligation to induce expression of CD80 and MHC class II by exposure of these cells *in vitro* to IFN-γ. In PBC, in liver allograft rejection, and in chronic GVHD, bile duct epithelia are penetrated by T cells, which mediate focal necrosis and may destroy the epithelium. Although poorly understood, the mechanisms that regulate leukocyte recruitment to portal tracts are likely to include locally secreted chemotactic factors. Chemotactic activity has been detected in bile during acute allograft rejection, although the precise nature of these cytokines and their cellular origins is not known [57]. In PBC, it has been shown immunohistochemically by several laboratories that the major autoantigen in PBC (PDC-E2) or a PDC-E2 mimic is expressed aberrantly on bile duct epithelium [58]. Nonetheless, a unique BEC antigen has not yet been identified.

Conclusion

The involvement of bile in the circulatory pathway of immunoglobulins and the presence of immunoglobulin in bile, together with the fact that biliary epithelial cells have functions characteristic of immunologic cells, remain intriguing topics. Although much progress has been made on the role that BECs play in the pathology of so-called vanishing bile duct diseases, there are still many areas requiring further investigation. BECs appear to be central participants in the pathology of PBC, PSC, chronic GVHD, and liver allograft rejection. However, it remains to be established whether the immunologic activity observed in BECs is a consequence of some other yet unknown injury, or if the BECs are the initiators of pathology in these diseases.

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[9] MHC = major histocompatibility complex
[10] CTLs = cytotoxic T lymphocytes
[11] PBC = primary biliary cirrhosis
[12] VCAM-1 = vascular cell adhesion molecule-1
[13] LFA-1 = leukocyte function-associated antigen-1
[14] PSC = primary sclerosing cholangitis
[15] GVHD = graft-vs-host disease

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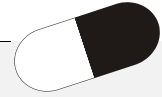
Capsule



Co-stimulation: building an immunological synapse

A central event in the development of immunity is the activation of the T cell. At the center of this process is the T cell receptor (TCR), which triggers activation by a specific interaction with antigen [usually a foreign peptide bound to, or "presented by," the organism's own major histocompatibility complex (MHC) molecule on the surface of an antigen-presenting cell]. Because of the small size of the TCR, its low affinity toward antigen, and the limited numbers of antigens on the antigen-presenting cell, an elaborate adhesion complex must be formed to allow the TCR to contact, sample, and then be activated by the rare antigenic ligands. This specialized contact area has been termed the immunological synapse.

Efficient T cell activation requires engagement of at least two types of T cell surface receptors. This phenomenon has been interpreted in terms of a "two-signal model," which proposes that T cell activation requires one signal from the TCR and a second signal from a "co-stimulator" molecule. Although many molecules have been implicated as co-stimulators, CD28 has become the archetype for co-stimulatory molecules. Engagement of CD28 either by its ligand on the antigen-presenting cell [B7 (CD8O)], or by antibody, can strongly enhance TCR signaling responses. Although current models suggest that CD28 functions as a specific activator of the Jun kinase JNK or the nuclear transcription factor NF-kB, CD28 engagement by itself is not sufficient.

In a recent report, Wulfing and Davis demonstrated a novel mechanism for co-stimulation in forming the immune synapse. They show that co-stimulation initiates active directional transport of protein and lipid domains to the area of cell–cell contact. This transport process requires myosin and correlates with enhanced, as well as sustained, signaling — a hallmark of co-stimulation. In these experiments, directed transport could be stimulated by either CD28 or LFA-1 engagement, but occurred most efficiently when both were engaged together. Wulfing and Davis propose that co-stimulation works by activating an actin–myosin driven transport process that delivers receptors and signaling complexes to the contact area. In this study, however, the transport process appears to be indiscriminate, and the key cargo was not clearly identified. Most recently, Viola et al. showed that the cargo for the actin-based transport mechanism is the 70 nm diameter lipid rafts also referred to as caveolae or detergent-insoluble glycolipid domains. The rafts are initially distributed evenly on the T cell surface and remain so after engagement of TCRs by beads coated with antibody to the TCR. Remarkably, engagement of CD28, together with the TCR, recruits essentially all the rafts to the contact area. This correlates with an increased lifetime for tyrosine phosphate, which may occur through phosphatase exclusion and increased consumption of the Lck kinase, indicative of greater tyrosine kinase activation. It has recently been suggested that engaged TCRs migrate into rafts. Viola et al. now demonstrate that it is the rafts that migrate to engaged TCRs and CD28.

These studies suggest that co-stimulation modulates the signaling environment around the engaged TCRs. Rafts are rich in kinases and adapter molecules which are required for T cell activation. In addition, the rafts' topological features are also compatible with their promoting sustained TCR engagement. Because glycolipids and small glycophosphatidylinositol-anchored molecules such as CD59, DAF, alkaline phosphatase and Thy-1 are concentrated in rafts, these domains may represent regions of reduced steric hindrance where interaction of the short TCR and MHC would be favored. In addition, cholesterol in lipid rafts may increase membrane rigidity and enhance the affinity of membrane protein interactions.

The co-stimulation-initiated transport mechanism appears capable of transporting anything linked to actin. Since both positive and negative regulators of T cell signaling may be associated with the actin cytoskeleton, how does the process achieve selectivity? One type of selectivity is demonstrated in the extreme by the movies of Wulfing and Davis: size selectivity. Large beads are transported to the edge of the synapse, but are excluded as they are too big to enter. On a molecular scale, integrins, the group of adhesion molecules that includes LFA-1, can generate effective occlusive barriers that exclude large molecules from contact areas. Other researchers have proposed that molecules such as CD2, which interact with ligands to generate very small gaps (<15 nm) between apposed membranes, are also involved in large-molecule exclusion. If the actin-based transport process can convey molecules to the center of the immunological synapse, then these barriers could be conceived of as molecular filters allowing only small molecules to enter the contact area, while excluding molecules with larger ectodomains.

The conventional view of T cell signaling held that each type of receptor generates its own distinct signal or has its own "voice." This collection of independent voices from the surface was then harmonized (integrated) in the nucleus to regulate transcription. The new concept that is emerging suggests that the immune synapse functions to tune, adjust, and amplify a single voice, the signal transduced by the TCR. T cell receptor signaling is intimately associated with contact formation because extended cell contact is required to maintain TCR engagement. This new concept is supported by recent studies of molecular organization of components in the immunological synapse and by the demonstration that specific transport mechanisms organize the contact area. Although we do not yet know whether CD28 and LFA-1 produce specific biochemical signals to initiate this transport process, a new paradigm for immunological co-stimulation is emerging that is built around the central role of contact formation in T cell activation.

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