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BASIC AND TRANSLATIONAL—LIVER

Mouse and Human Liver Contain Immunoglobulin A–Secreting Cells Originating From Peyer's Patches and Directed Against Intestinal Antigens

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BACKGROUND & AIMS: The liver receives blood from the gastrointestinal tract through the portal vein, and thereby is exposed continuously to dietary antigens and commensal bacteria. Alcoholic liver disease (ALD) is associated with intestinal dysbiosis, increased intestinal permeability, release of microbes into the portal circulation, and increased serum levels and liver deposits of IgA. We characterized B-cell production of IgA in livers of mice at homeostasis, after oral immunization, in a mouse model of ALD and in human liver samples. **METHODS:** We performed studies with Balb/c and C57BL/6-Ly5.1 mice, as well as transgenic mice (quasimonoclonal, activation-induced [cytidine] deaminase–Cre-tamoxifen-dependent estrogen receptor 2 [ERT2], Blimp-1-green fluorescent protein [GFP]). C57BL/6-Ly5.1 mice were fed chronic plus binge ethanol to create a model of ALD. Some mice also were given repeated injections of FTY720, which prevents egress of IgA-secreting cells from Peyer's patches. We obtained nontumor liver tissues from patients with colorectal carcinoma undergoing surgery for liver metastases or hepatocellular carcinoma. B cells were isolated from mouse and human liver tissues and analyzed by flow cytometry and enzyme-linked ImmunoSpot (ELISpot). In wild-type and transgenic mice, we traced newly generated IgA-secreting cells at steady state and after oral immunization with 4-hydroxy-3-nitrophenylacetyl (NP)-Ficoll or cholera toxin. IgA responses were also evaluated in our model of ALD. **RESULTS:** Livers of control mice contained proliferative plasmablasts that originated from Peyer's patches and produced IgAs reactive to commensal bacteria. After oral immunization with cholera toxin or a thymus-independent antigen, a substantial number of antigen-specific IgA-secreting cells was found in the liver. Mice fed ethanol had features of hepatitis and increased numbers of IgA-secreting cells in liver, compared with mice given control diets, as well as higher levels of serum IgA and IgA deposits in liver sinusoids. Injection of FTY720 during ethanol feeding reduced liver and serum levels of IgA and IgA deposits in liver and prevented liver injury. Human liver tissues contained a significant proportion of IgA-producing plasma cells that shared phenotypic and functional attributes with those from mouse liver, including reactivity to commensal bacteria. **CONCLUSIONS:** Based on studies of mice and human liver tissues, we found the liver to be a site of IgA production by B cells, derived from gut-associated lymphoid tissues. These IgAs react with commensal bacteria and oral antigens. Livers from mice with ethanol-induced injury contain increased numbers of IgA-secreting cells and have IgA deposits in sinusoids. IgAs in the liver could mediate clearance of gut-derived antigens that arrive through portal circulation at homeostasis and protect these organs from pathogens.

Keywords: Microbiota; Humoral Response; S1PR1; Hepatic.

Because of its unique vasculature system and anatomic localization, the liver receives the majority of blood supply from the gastrointestinal tract through the portal vein, and thus faces continuous exposure to dietary antigens and bacterial components. Besides its essential metabolic functions, the liver prevents systemic dissemination of gut-derived endotoxins and also appears essential for immune tolerance to intestinal antigens,[1–3] a role long attributed solely to gut-associated lymphoid tissues (GALT). Thus, the gut and the liver share a microenvironment prone to tolerance and theoretically also to induction of IgA response owing to the presence of IgA-prone cytokines and tolerogenic antigen-presenting cells.[4] However, whether the liver indeed contributes to the intestinal IgA response remains unclear. Moreover, although the biology of most hepatic immune cell subsets has been amply documented, not much is know regarding liver B-cell distribution and biology.[5]

Intestinal IgAs limit bacterial translocation and systemic dissemination, neutralize pathogenic microorganisms and

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Abbreviations used in this paper: **AID, activation-induced (cytidine) deaminase; ALD, alcoholic liver disease; BM, bone marrow; BrdU, bromodeoxyuridine; CCR, CC family chemokine receptors; CSR, class-switch recombination; CT, cholera toxin; CXCR, CXC family chemokine receptors; EYFP, enhanced yellow fluorescent protein; GALT, gut-associated lymphoid tissue; LNPC, liver nonparenchymal cell; LP, lamina propria; MHC, major histocompatibility compex; mLN, mesenteric lymph node; PB, plasmablast; PBS, phosphate-buffered saline; PC, plasma cell; PP, Peyer's patch; QM, quasimonoclonal; SFC, spot-forming cell; SI LP, small intestine lamina propria; TD, T-cell dependent; TI, T-cell independent.**

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associated toxins, and maintain diversity of the gut commensals.[6] Gut IgA plasma cells (PCs) originate from B cells activated in GALT in response to T-cell dependent (TD) or T-cell independent (TI) intestinal antigens. B cells massively expand in Peyer's patches (PP), undergo IgA class-switch recombination (CSR) and somatic hypermutation under the control of activation-induced (cytidine) deaminase (AID) and differentiate into plasmablasts (PBs), producing high-affinity (TD antigen) or low-affinity "natural" (TI antigen) IgA.[7] PBs then transit to mesenteric lymph nodes (mLNs), where they further differentiate and receive signals to home to the intestinal lamina propria (LP), and to a lesser extent in spleen and bone marrow (BM), where they further mature into antibody-producing PCs.

The first link between the liver and intestinal humoral immunity was illustrated by the hepatobiliary transport of IgA, which clears free IgAs and IgA immune complexes from blood, and fuels the duodenum with functional polymeric IgAs via the bile.[8] That a significant part of biliary IgAs might originate from liver was suggested by the presence of immunoglobulin-secreting cells in rat liver parenchyma[9] and human biliary epithelium.[10] Moreover, circulating IgAs were proposed to contribute to endotoxin clearance in the liver, mediated by Kupffer cells.[11] Interestingly, alcoholic liver disease (ALD), characterized by dysbiosis and increased intestinal permeability leading to abnormal bacterial translocation in portal circulation,[12] is associated with increased serum IgA levels and liver IgA deposits.[13] However, the origin of these IgA disorders and whether IgA could participate in liver damage remains poorly understood.

In this study, we show that the liver constitutes an effector site for IgA responses because it hosts PBs/PCs originating from PP and secreting IgAs against gut commensal bacteria and orally delivered antigens. Moreover, we document in a model of ALD that accumulation of IgA PBs in the liver is linked to IgA disorders, and that liver injury is prevented by a drug blocking PP-derived IgA PB migration to the liver.

Materials and Methods

Mice

Balb/c and C57BL/6-Ly5.1 mice were purchased from Charles River Laboratories (L'Arbresle, France). Quasimonoclonal (QM) mice[14] were kindly provided by M. Cascalho (Ann Arbor, MI). AID-Cre-ERT2 transgenic mice[15] were obtained from C.A. Reynaud (Paris, France) and were crossed with ROSA26-loxP-enhanced yellow fluorescent protein (EYFP) mice (Jackson Laboratory, Bar Harbor, ME). Their progeny received 10 mg tamoxifen (Sigma Aldrich, St Louis, MO) orally on 2 consecutive days. Blimp-1-GFP mice[16] were kindly provided by S. Nutt (Victoria, Australia). In some experiments, mice received daily intraperitoneal injections of 25 μg FTY720 (Sigma Aldrich). Except Balb/c mice, all mice were bred under specific pathogen-free conditions in the Plateau de Biologie Expérimentale de la Souris (Ecole Normale Supérieure, Lyon, France) and were used at 10–16 weeks of age. All mouse studies were approved by the local ethics committee (CECCAPP Lyon, registered by the French National Ethics Committee of Animal Experimentation under no. 15) in accordance with European guidelines for animal experiments. Except when indicated, Balb/c mice were used.

Human Biological Samples

Macroscopically and histologically healthy portions of human liver were obtained from patients with colorectal carcinoma undergoing liver metastasis resection at the Centre Léon Bérard and Centre Hospitalier Lyon-Sud (Lyon, France). Nontumoral tissue from resected liver of hepatocellular carcinoma patients was obtained from Croix-Rousse Hospital (Lyon, France). All patients provided informed consent and protocols were approved by the hospital ethics committee. Normal sera were obtained from healthy donors at the Etablissement Français du Sang Rhône-Alpes Auvergne.

Liver Nonparenchymal Cell Preparation

Mouse liver nonparenchymal cells (LNPCs) were obtained by mechanical dissociation using GentleMACS (Miltenyi Biotec, Paris, France) of cold PBS-perfused liver, followed by 40%/80% Percoll density gradient centrifugation (GE Healthcare, Aulnay-Sous-Bois, France). Human LNPCs were obtained from perfused liver fragments by a 30- to 40-minute enzymatic digestion with 0.1 mg/mL DNAse I and 1 mg/mL collagenase IV (Worthington, Lakewood, NJ), followed by a Percoll gradient.

Oral Immunization and Antigen-Specific B Cell In Vivo Tracking

Mice were immunized orally by gavage with 5 mg NP_{77}-AECM-FICOLL (NP-Ficoll) (Biosearch Technologies, Novato, CA) and/or 10 μg cholera toxin (CT) (List Biological Laboratories, Campbell, CA) in $NaHCO_3$ 0.2 mol/L, pH 8.3. For adoptive transfer experiments, spleen cells from QM mice (Ly5.2) were stained with CellTrace Violet (Life Technologies, Carlsbad, CA) and injected intravenously (10^7) into C57BL/6-Ly5.1 recipients just before oral immunization.

Mouse Model of Chronic Alcohol Consumption

We used the recently described chronic-binge ethanol feeding model.[24] Briefly, after 5 days of acclimatization to feeding tubes and the Lieber-DeCarli liquid diet (Bio-Serv, FrenchTown, NJ), C57BL/6-Ly5.1 mice were pair-fed for 10 days with either control diet or 5% ethanol-containing liquid diet. At day 10, mice received a single binge of ethanol (5 g/kg) or isocaloric dextrin-maltose and were killed 9 hours later. Serum aspartate aminotransferase and alanine aminotransferase were measured with an Abbott Architect c16000 chemistry analyzer (Rungis, France).

Analysis of IgA Coating of Fecal Bacteria by Flow Cytometry

Mouse fecal bacteria were isolated as described elsewhere[23] and were incubated for 1 hour on ice with sera, intestinal washes, or LNPC culture supernatant diluted in phosphate-buffered saline (PBS) + protease inhibitor (cOmplete protease inhibitor, Roche, Meylan, France) to reach a concentration of 1 μg/mL of IgAs. After washings, bacteria were incubated with anti-IgA–biotin (Southern Biotech, Birmingham,