Unique Aspects of the Neonatal Immune System Provide Clues to the Pathogenesis of Biliary Atresia

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- Financial Disclosure:
  No financial relationships to disclose relevant to the presentation
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  The presentation does not include discussion of off-label use of medications

Objectives

- Update on outcomes in biliary atresia
- Highlight unique aspects of neonatal adaptive immunity that provide clues to biliary atresia pathogenesis
  - Th1 cellular immunity (IFN-γ)
  - Th17 cellular immunity (IL-17)
  - Antigen presenting cell function
  - Regulatory T cells
Biliary Atresia (BA)

- Progressive inflammatory sclerosing process of biliary tract with obstruction by age 3 months
- Incidence per live births: 1:5,600 (Taiwan), 1:18,000 (Europe), 1:12,000 (U.S.)
- BA types: 1) Isolated (~85% of BA)
  2) BA associated with splenic malformation
  3) Other anomalies
  4) Cystic BA

Theories of BA Pathogenesis

- Defective morphogenesis
- Cholangiotropic virus infection (reovirus, rotavirus, CMV)
- Toxin
- Autoimmune cholangiopathy
- Vascular injury/ischemia

Virus-Induced, Progressive Autoreactive T cell-Mediated Injury of Bile Duct Epithelia (BDE)

Adapted from Mack, CL. Sem Liver Dis 2007
Virus-Induced, Progressive Autoreactive T cell-Mediated Injury of Bile Duct Epithelia (BDE)

Presentation viral Ag
T cell activation

Presentation self BDE autoreactive T cell activation

Phagocytosis of BDE

Adapted from Mack, CL, Sem Le Dis 2017

Survival with Native Liver
Post-Kasai-Denver:
1972-1996
(N=266)

Survival with Native Liver
Post-Kasai-France:
1986-2009
(N=1,044)


Chardot et al. Jnl Hepatol 2013
Medical Status of Children with BA Surviving with Native Livers (ChiLDReN)

- Analysis of outcome 10.5 years after successful Kasai (range 5-18 yrs) (N=219)
- Chronic liver disease: 90%
- "Ideal" outcome: 1.8% of patients
  - normal liver tests
  - no signs of chronic liver disease
  - no liver-specific medications
  - normal quality of life

Ng et al. J Pediatr 2014

Adult Outcomes of BA with Native Liver

- Analysis of outcome 25 years after successful Kasai (range 18-46 yrs) (N=22)
- Cirrhosis and portal hypertension: 95%
- Jaundice: 50%
- Bacterial cholangitis: 50%
- Features of sclerosing cholangitis: 60%

Kumagi et al. Liv International 2012

Unique aspects of neonatal cellular (T cell) adaptive immunity that provide clues to disease pathogenesis
"The neonate should not be considered simply immunodeficient, but rather immunodiverse, with the ability to generate adult-like responses depending on specific circumstances."

Becky Adkins, Univ. of Miami

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**Th1 Cell Immunity (IFN-γ) in Neonates**

IFN-γ: cellular immunity (infection), inflammation, autoimmunity
Generation of neonatal Th1 response

**A**
- High dose virus
- Adult
- Newborn

**B**
- Low dose virus
- Newborn

**T cell Response**
- Induction of virus specific CTL and IFN-γ responses (Th1-Type 1 Response)
- No virus specific CTL or IFN-γ responses
- Induction of IL-4 (Type 2 Response) Th2

**Inflammatory Milieu in BA**
- Portal tract periductal infiltrates
- Extrahepatic bile duct chronic inflammation

- Activated CD4+ and CD8+ T cells producing Th1 cytokines (IL-2, IFN-γ, TNF-α)

Davenport et al., J Pediatr Surg 2001
Bezerra et al., Lancet 2002
Mack et al., Ped Res 2004

**Rhesus Rotavirus (RRV)- Induced Mouse Model of BA**
- 12 hr. old BALB
- RRV i.p.
- 1 week old
  - Jaundice, RRV present
- 2 week old
  - Biliary obstruction, RRV cleared

- Extrahepatic bile duct
- Portal tract

- saline (control)
- RRV (BA)

- Th1 cell-mediated periductal inflammation and obstruction
IFN-γ Necessary for Biliary Obstruction in BA Mice

- IFN-γ knockout mice are protected from biliary disease
- Mice depleted of IFN-γ producing CD8+ T cells are protected from biliary disease

Autoimmunity in BA mice: Bile Duct Epithelial (BDE) Proteins Stimulate Autoreactive CD4+ (IFN-γ) T Cells

Liver T cells

The NSP4 viral protein is responsible for biliary tract injury in biliary atresia
CD8⁺ IFN-γ-producing T cells in GST-NSP4 injected mice

NSP4-specific CD8⁺ T cells (IFN-γ⁺) cross-reactive to bile duct epithelial proteins

Th17 Cell Immunity (IL-17) in Neonates

IL-17: major pathogenic cytokine contributing to inflammation and autoimmunity
Enhanced pro-inflammatory Th17 responses in human neonates


Th17 cellular infiltrates and outcome in BA


Murine BA: γδ T cells produce IL-17

Klemann et al. Gastroenterol 2015
Murine BA: γδ T cells produce IL-17

IL-17 blockade associated with decreased biliary obstruction

Increase Th17 signature in human BA

Antigen Presenting Cell Function in Neonates

Professional APCs: dendritic cells, macrophages, B cells
Diminished function of neonatal APCs

- Neonatal APCs have low levels of MHC (signal 1) and co-stimulatory molecules (B7-1, B7-2) (signal 2)
- Neonatal dendritic cells have lower levels of cytokine production (signal 3)
- Neonatal regulatory T cells inhibit APC functions
- However… certain conditions can stimulate adult-like antigen presentation/T cell activation


Neonatal liver plasmacytoid dendritic cells (pDC) have adult levels of co-stimulation

Conventional DCs

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Plasmacytoid DCs

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Saxena et al. Sci Transl Med 2011

Upregulation of MHC, B72 and CD40 on liver B cells in murine BA (age 7 days)

Liver B cells: red- control mice; blue- RRV-induced BA mice

MHC1 MHC2 B7-1 B7-2 CD40 CD69

Livex BSS Livex RRV Spleen BSS Spleen RRV

Traxinger et al. MUSLD 2015
Role of B Cells in Bile Duct Injury in Mouse BA

- Ig-α− mice: Loss of B cell receptor expression
  - Defective antigen presentation
  - Defective immunoglobulin production

B Cell Deficient Mice are Protected from Biliary Obstruction

B Cell Deficient Mice have Significantly Decreased Th1 cytokines
Regulatory T cells (Tregs)

- CD4+ CD25+ FoxP3+
- Suppresses pathogen-mediated inflammation
- Inhibits CD4+ T cell-mediated autoimmune responses

High numbers of Tregs in neonate

Grindebacke et al., J Immunol 2009
Neonatal Tregs have potent suppressive function

T cell suppression assay (activated T cells with or w/o culture with Tregs)

Brindley et al. Hepatology 2012 (ChiLDReN)
Tucker et al. JHL Hepatol 2013

Treg Deficiencies in BA

Adoptive Transfer of Naïve Tregs into BA mice Improves Survival

Tucker et al. JHL Hepatol 2013
Lages et al. Hepatology 2012
Summary

- Evidence exists for exaggerated Th1 and Th17 responses contributing to bile duct injury in BA.
- The persistent Th1 and Th17 responses are due to highly activated liver APCs and dysfunctional Tregs.
- Understanding mechanisms of immune-mediated injury and fibrogenesis in BA will lead to targeted therapies.

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