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

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Disclosures

- Financial Disclosure:
  No financial relationships to disclose relevant to the presentation
- Off-label/ investigational use of medications:
  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)

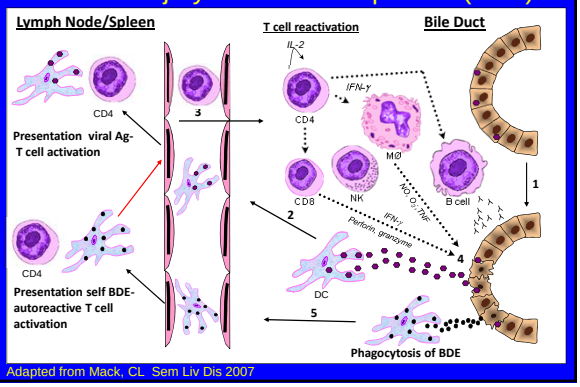
- Progressive inflammatory sclerosing process of biliary tract with obstruction by age 3 months
- Incidence per live births- 1:5,600 (Taiwan), 1:18,000 (Europe), 1:12,000 (U.S.)
- BA types: 1) Isolated (~85% of BA)

2) BA associated with splenic malformation
3) Other anomalies
4) Cystic BA

Theories of BA Pathogenesis

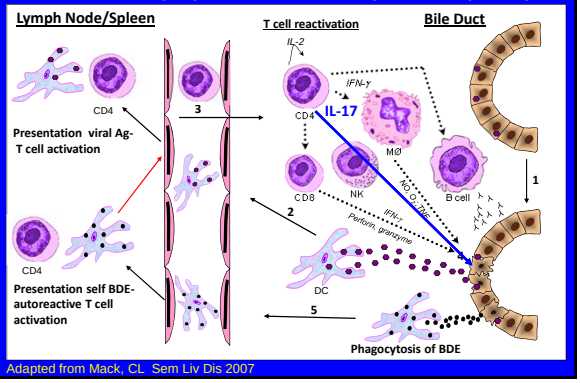
- Defective morphogenesis
- Cholangiotropic virus infection (reovirus, rotavirus, CMV)
- Toxin
- Autoimmune cholangiopathy
- Vascular injury/ischemia

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



Adapted from Mack, CL Sem Liv Dis 2007

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



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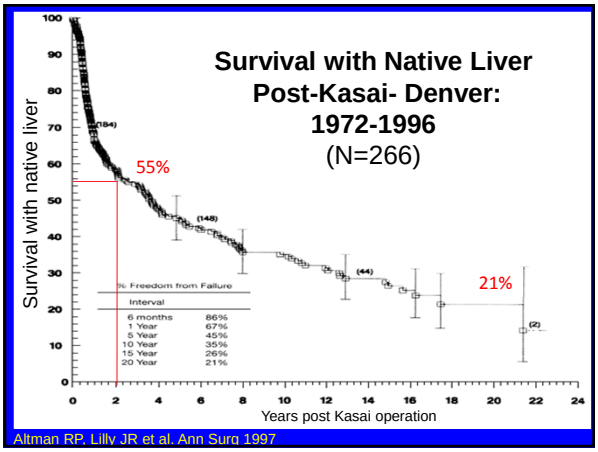
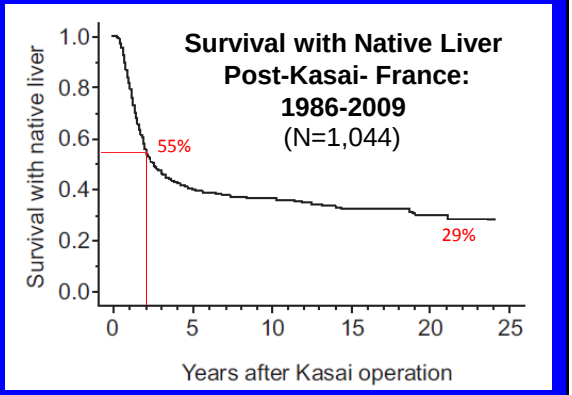
Survival with Native Liver Post-Kasai- Denver: 1972-1996 (N=266)



Altman RP, Lilly JR et al. Ann Surg 1997

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



Chardot et al. Jrl 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

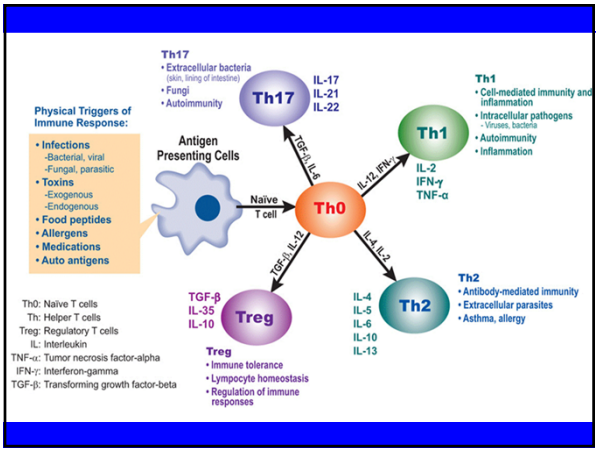
- Analysis of outcome 25 years after successful Kasai (range 18-46 yrs) (N=22)
- Cirrhosis and portal hypertension: 95%
- Jaundice: 50%
- Bacterial cholangitis: 50%
- Features of sclerosing cholangitis: 60%

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"

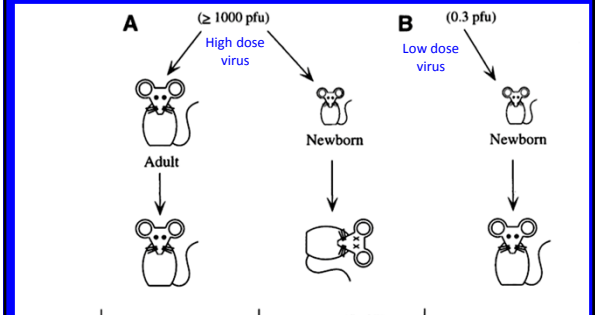
Becky Adkins, Univ. of Miami



Th1 Cell Immunity (IFN-γ) in Neonates

IFN-γ: cellular immunity (infection), inflammation, autoimmunity

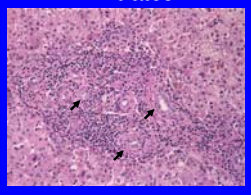
Generation of neonatal Th1 response



Hoffman et al. J Neuroimmun 1981

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

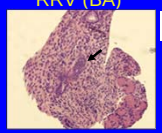
12 hr. old BALB

1 week old → Jaundice, RRV present

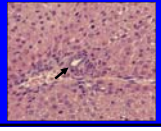
2 week old → biliary obstruction, RRV cleared

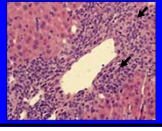
saline (control) | RRV (BA)

Extrahepatic bile duct



Portal tract



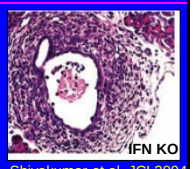


bile ducts

- Th1 cell-mediated periductal inflammation and obstruction

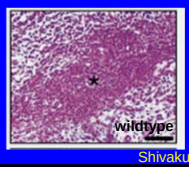
IFN-γ Necessary for Biliary Obstruction in BA Mice

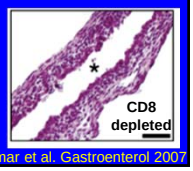
- IFN-γ knockout mice are protected from biliary disease



Shivakumar et al. JCI 2004

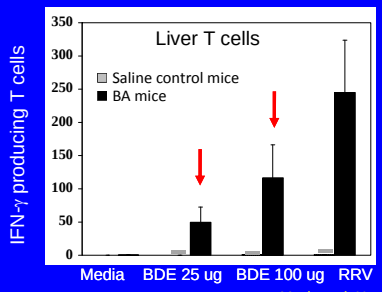
- Mice depleted of IFN-γ producing $CD8^+$ T cells are protected from biliary disease





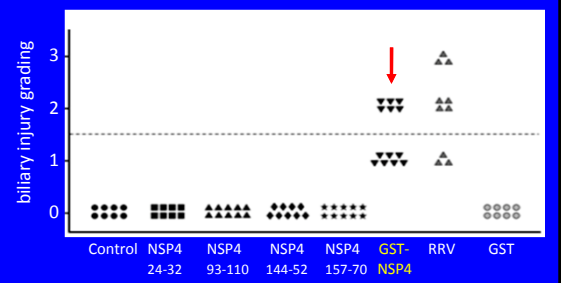
Shivakumar et al. Gastroenterol 2007

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



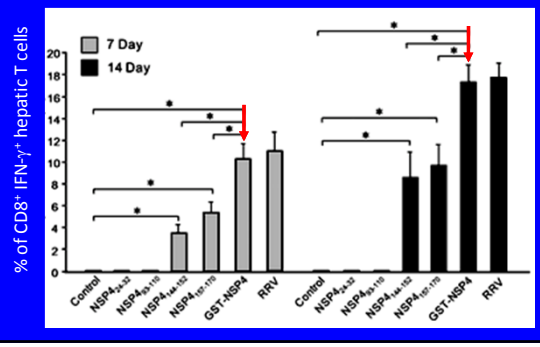
Mack et al. Hepatology 2006

The NSP4 viral protein is responsible for biliary tract injury in biliary atresia

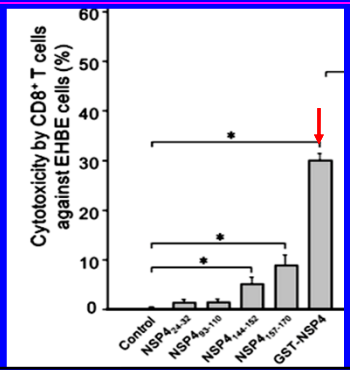


Zheng et al. Amer J Physiol Gastrointest Liver Physiol 2014

CD8+ IFN-γ-producing T cells in GST-NSP4 injected mice



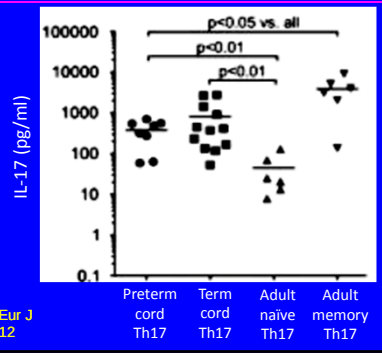
NSP4-specific CD8+ T cells (IFN-γ+) cross-reactive to bile duct epithelial proteins



Th17 Cell Immunity (IL-17) in Neonates

IL-17: major pathogenic cytokine contributing to inflammation and autoimmunity

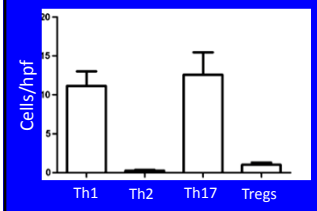
Enhanced pro-inflammatory Th17 responses in human neonates



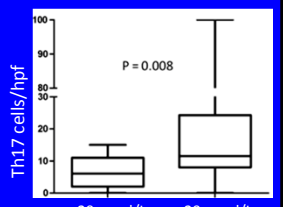
Black et al. Eur J Immunol 2012

Th17 cellular infiltrates and outcome in BA

Quantification of portal tract T cells in biliary atresia



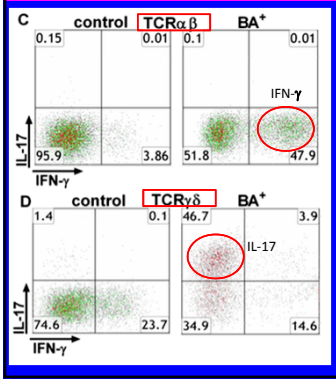
Th17 cells and poor outcome



Bilirubin 6 months post-Kasai

Hill et al. J Ped Surg 2015

Murine BA: γδ T cells produce IL-17



Klemann et al. Gastroenterol 2015

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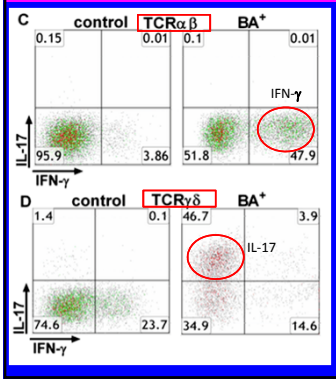
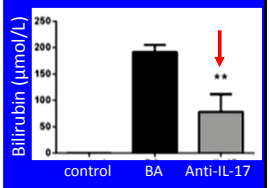
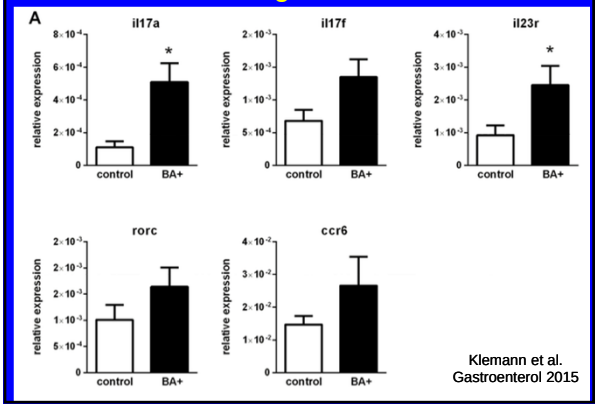


IL-17 blockade associated with decreased biliary obstruction



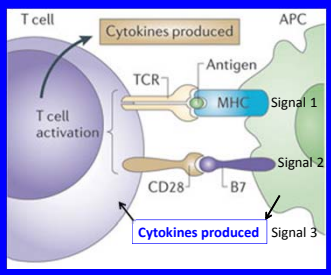
Klemann et al. Gastroenterol 2015

Increase Th17 signature in human BA



Klemann et al. Gastroenterol 2015

Antigen Presenting Cell Function in Neonates



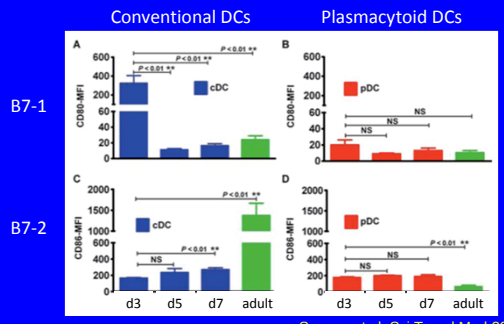
Professional APCs: dendritic cells, macrophages, B cells

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

Villila et al. Clin Immun 2006
Marchant et al. Clin Exp Immun 2005

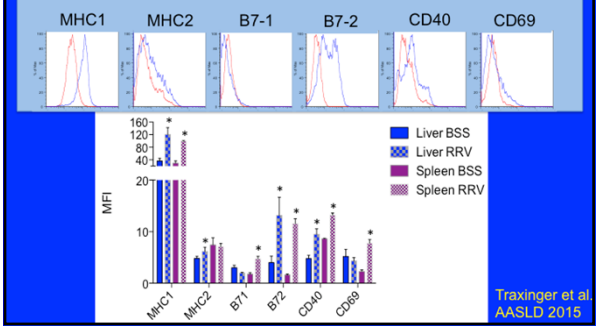
Neonatal liver plasmacytoid dendritic cells (pDC) have adult levels of co-stimulation



Saxena et al. Sci Transl Med 2011

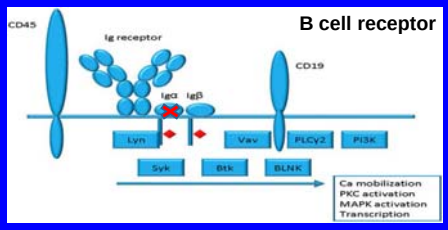
Upregulation of MHC, B72 and CD40 on liver B cells in murine BA (age 7 days)

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



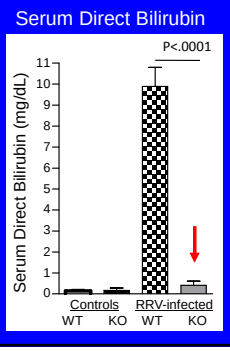
Traxinger et al. AASLD 2015

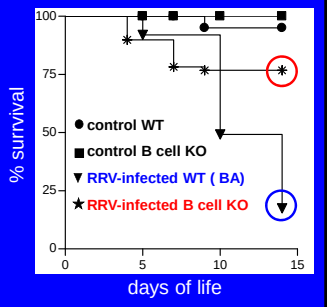
Role of B Cells in Bile Duct Injury in Mouse BA



- Ig-$\alpha^{-/-}$ mice: Loss of B cell receptor expression
  - Defective antigen presentation
  - Defective immunoglobulin production

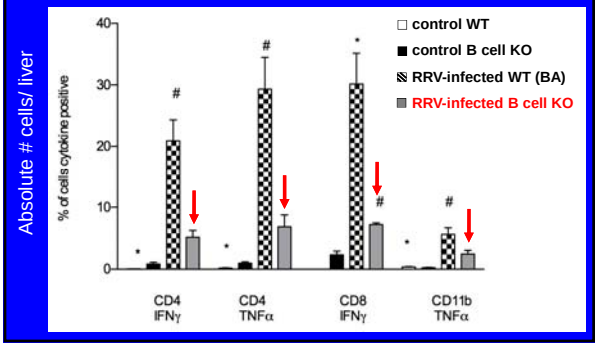
B Cell Deficient Mice are Protected from Biliary Obstruction





Feldman et al., PLoSOne 2013

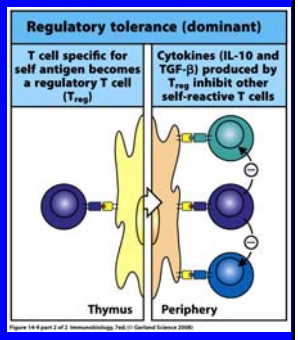
B Cell Deficient Mice have Significantly Decreased Th1 cytokines



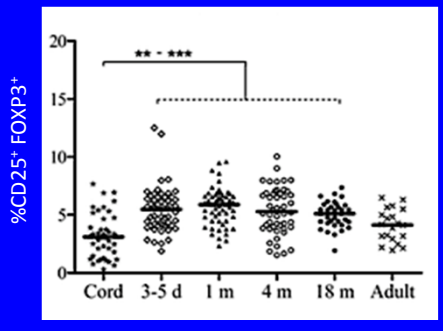
Regulatory T cells (Tregs)

Regulatory T Cells (Tregs)

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



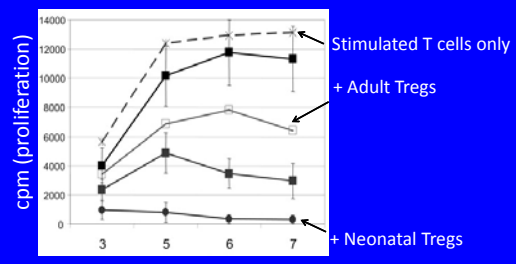
High numbers of Tregs in neonate



Grindebacke et al., J Immunol 2009

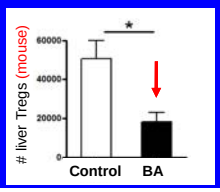
Neonatal Tregs have potent suppressive function

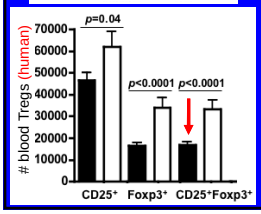
T cell suppression assay (activated T cells with or w/o culture with Tregs)



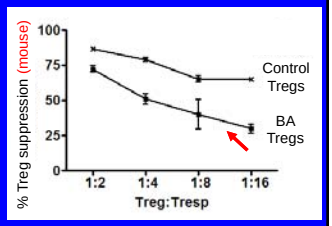
Godfrey et al. Blood 2005

Treg Deficiencies in BA





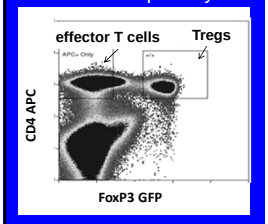
Treg Suppression Assay

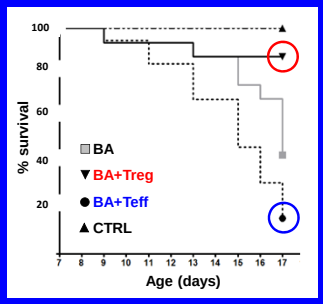


Brindley et al. Hepatology 2012 (ChiLDReN)
Tucker et al. Jrl Hepatol 2013

Adoptive Transfer of Naïve Tregs into BA mice Improves Survival

Naïve donor splenocytes

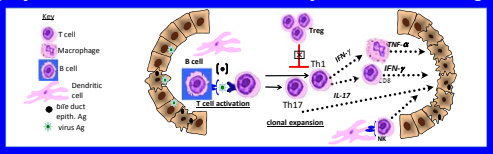




Tucker et al. Jrl Hepatol 2013
Lages et al. Hepatology 2012

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

Mack Lab- contributors
Past: Rebecca M. Tucker, PhD
Stephen Brindley
Erika Fenner
Amy Feldman, MD
Present: Brianna Traxinger
Jon Roach, MD
Dania Molla Hosseini, MD
Asokan Rengasamy, PhD

Integrated Dept of Immunology
University of Colorado
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Ronald Gill, PhD

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