Batf3 Dependent Dendritic Cells are Crucial for Development of Primary Biliary Cholangitis

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Primary Biliary Cholangitis (PBC)

- Progressive autoimmune cholestatic liver disease
- Previous studies exhibited major contribution for CD8+ T cells and for the cytokine IL-12 in the pathogenesis of PBC
AMA targets Pyruvate dehydrogenase E2 complex (PDC-E2)

AMA-positive individuals, are very likely to develop PBC

PDC-specific CD8+ T cells identified in the livers of the patients
Genetic factors/susceptibility

• A concordance rate of 63% in monozygotic twins and common in first degree relatives

• Polymorphisms of genes involved in innate or adaptive immunity:
  – HLA class II
  – Interleukin 12 alpha (IL12A) locus & SNP Interleukin 12 receptor beta 2
  – IRF5
  – CD80
  – STAT4
  – SPIB
  – TNFα
  – CTLA-4
Macrophages and Dendritic cells origin

- primitive macrophages
- HSC
- MDP
- monocytes
- monocyte-derived Macrophages/DCs
- CDP
- Pre-DC
- PDC
- CD4+
  - IRF4
- CD8α+ CD103+
  - IRF8
  - BATF3
- FLT3-dependent classical DCs

- tissue-resident Macrophages
  - long-lived, self-renewing
  - Microglia
  - Kupffer cells

- classical DCs
BATF3 (CD103+CD11b-) Dendritic Cells

- Efficiently perform Cross-presentation of antigen to CD8+ T cells

- IL-12 production by this cells is important in defense against intracellular pathogens

Kamphorst AO, et al. JI 2010
Dendritic Cells in PBC

- CD11c is highly expressed in hepatic granulomas from PBC liver samples
  You Z. et al, J Autoimmun 2012

- DCs were identified in portal tracts and in bile duct epithelium, significantly higher than in other chronic inflammatory liver conditions

Solid data evaluating the importance and function of these cells in the in-vivo context is still missing
PBC – murine xenobiotic induced model

- AMA reacts against the 2-octynoic acid-modified PDC- E2 peptide

- A murine model for PBC based on immunization of mice with 2-octynoic acid conjugated to bovine serum albumin (2-OA BSA)

**Day 1**- Injection of 2OA-BSA emulsified with complete adjuvant and 100ng Pertussis toxin

**Day 2**- Injection of 100ng Pertussis toxin

**Day 14**- Injection of 2OA-BSA emulsified with incomplete adjuvant

8-12 weeks
Mice

- **Batf3 KO mice** - Specifically lack only CD103+CD11b- DCs

- **WT mice**

  - **Day 1** - Injection of 2OA-BSA emulsified with complete adjuvant and 100ng Pertussis toxin
  - **Day 2** - Injection of 100ng Pertussis toxin
  - **Day 14** - Injection of 2OA-BSA emulsified with incomplete adjuvant

8-12 weeks
Hepatic DCs in Autoimmune Cholangitis

Gated on CD45+ cells

WT

CD11c

CD64

CD11b

MHC II

CD11c

CD64

CD11b

MHC II

CD11c

CD64

CD11b

BATF3−/−

CD11c

CD64

CD11b

MHC II

CD11c

CD64

CD11b

Gated on CD45+ cells

MHCI

CD64

CD11b

CD103

CD11c

CD64

CD11b

CD103

Gated on CD45+ cells

MHCI

CD64

CD11b

CD103

CD11c

CD64

CD11b

CD103

Gated on CD45+ cells
BATF3 KO mice are protected in the 2OA-BSA model

Histological disease severity (0-8)

- Portal infiltrate
- Bile duct damage
- Bile duct loss
- Granulomas

Normal - 0
Mild - 1
Moderate - 2

Histology severity score

WT

Batf3⁻/⁻

p=0.02
CD4/CD8 hepatic T cell ratio

WT

BATF3 KO

CD4/CD8 T cells ratio

WT

Batf3−/−

p=0.0004
Cytokines / chemokines in the liver

- **TNFα**
  - Relative expression
  - WT: 1.25
  - Batf3^{-/-}: 0.55
  - p = 0.005

- **IFNγ**
  - Relative expression
  - WT: 1.05
  - Batf3^{-/-}: 0.7
  - p = 0.03

- **CCL2**
  - Relative expression
  - WT: 1.15
  - Batf3^{-/-}: 0.5
  - p = 0.01
In-vitro generation of Batf3-dependent DCs

WT

Batf3 KO
Future Plans

• Assess the capacity of CD103+ DCs in priming PDC-E2 specific CD8+ T cells in mice and humans

• Expression signature, Epigenetics and function of CD103+ DCs from PBC patients compared to healthy controls / other liver disorders
Conclusions:

• Batf3 dependent DCs are critical for the development of PBC

• May pave the road to immune based cell specific targeted therapeutic endeavors
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