The Circadian Clock: The pathophysiological Fundamentals

A glimpse at circadian immunity

Congrès Francophone d’Allergologie 2018

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No conflict of interest to disclose

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Benoit Pource, PhD
Prix Nobel de Médecine 2017
Horloge Biologique

Jeffery C Hall
Michael Rosbash
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Organisms rely on a well-conserved intrinsic clock

Processes that oscillate with a periodicity of 24 hours under constant conditions

Orchestrating the variation of physiological activity to the most appropriated time-window

Conserved (from bacteria to human)

Persist across a range of physiological temperature

Autonomous Endogenous

Entrained by external cues (light – food)

Anticipate environmental changes

Bass J Nature 2012
Circadian physiological clocks

CENTRAL CLOCK
Suprachiasmatic nucleus (SCN)

PERIPHERAL CLOCKS

Retinohypothalamic tract
Brain

Hyp^+ – SCN
Hyp^-

ACTH
SNS
PNS

Adrenal gland
Kidney

Glucocorticoid
Noradrenalin
Adrenalin

Heart
Spleen
Bone marrow
Stomach
Liver

Dibner C et al., Ann Rev Phys 2010
The molecular clock

- BMAL1
- CLOCK
- Rev-erbα, Rev-erbβ
- ROR
- Per1, Per2
- Cry1, Cry2
- Clock Control Genes

- Bmal1
- RORα
- NCoA
- ROE/RevRE
- CRY
- PER
- Bmal1
- Rev-erb-α
- NCoR
- CK2, CK1ε/δ
- AMPK

- Proteasomal degradation
- CK2, CK1ε/δ
- AMPK

- Rev-erb-α
- GSK3β
- Bmal1
- ROE/RevRE

- Retinoids
- RXRα
- vasculature
- liver

- Free fatty acids and derivatives

- Cortisol

- Estrogens

- Vasculature
- PPARα,γ
- Liver
- Free fatty acids and derivatives
- Retinoids

- Teboul M et al., J App Physiol, 2009
Physiology around the clock

- Bowel movement suppressed
- Melatonin secretion starts
- Highest body temperature
- Highest blood pressure
- Greatest muscle efficiency (Sk m. & heart)
- Fastest reaction time
- Best coordination
- Deepest sleep
- Body temperature
- In blood pressure
- Melatonin secretion stop
- Bowel movement likely
- High testosterone secretion
- High alertness
- Highest body temperature
- Fastest reaction time
- Greatest muscle efficiency (Sk m. & heart)
- Highest blood pressure

Bass J Nature 2012
Physiological role of the circadian clock

Vasculature
Heart rate
Blood pressure
Sleep
Brain activity
Bowel contraction
Reproductive system
Cell cycle
Body temperature
Immunity
Metabolism
Immunity around the clock

Immune System undergoing repair and regeneration

Active Phase

- Lethality to *D. pneumoniae*
- Proinflammatory cytokines with LPS
- TLR9 expression (ZT20)
- Leukocytes in tissue (ZT16)
- Clearance of *S typhimurium* (ZT18)

Resting Phase

- Lethality from sepsis
- Lethality to *D. pneumoniae*
- TLR9 expression (ZT8)
- Ly6C$^{hi}$ monocytes in circulation
- Highest leukocytes number in circulation
- Clearance of *S typhimurium*

Immune System poised for attack

Mann K et al., Science 2016
Associated-diseases to clock impairment

- Crohn’s Disease
- Type 2 diabetes
- Atherosclerosis
- Allergy
- Rheumatoid Arthritis
- Gouty
- Alzheimer’s Disease
- Cancer

Curtis AM et al., Immunity 2014; Videnovic A et al., Nat Rev Neur 2014
Associated-diseases to NLRP3 polymorphism

Curtis AM et al., Immunity 2014; Videnovic A et al., Nat Rev Neur 2014
The NLRP3 inflammasome pathway

Initiation – 1st signal
- LPS
- TLR4
- P2X7
- K^+
- Extracellular ATP

Activation – 2nd signal
- Efflux K^+
- Lysosome damage
- Lipids
- Crystals
- Lipids
- ADNmt
- Cardiolipin
- NEK7
- CtsB
- Pro-Casp1
- Pro-IL18
- Pro-IL1β
- IL-18
- IL-1β
- Casp1
- IL-18R
- IL-1R
- IL-18
- IL-1β
- IFNγ

Secretion
- Lymphocyte
- Th1, Th17

NLRP3 inflammasome pathway displays circadian rhythmicity

CD11b+ Peritoneal Exudate Cells (PEC)

Peritoneal lavage

Pourcet, Zecchin et al., Gastroenterology 2018
Circadian rhythmicity in primary macrophages

Pourcet, Zecchin *et al.*, Gastroenterology 2018
Rev-erbα physiological activities

- **T cells**
  - Pro-inflammatory
  - Th17 differentiation

- **Macrophage**
  - Pro-inflammatory M1 polarisation
  - Anti-inflammatory M2 polarisation
  - Circadian secretion of MCP-1 and IL-6

- **Skeletal muscle**
  - Mitochondrial biogenesis
  - Oxidative capacity
  - Endurance

- **Liver**
  - Lipoprotein metabolism (APOC3)
  - Neoglucogenesis (G6Pase, PEPCK)
  - Biliary acid metabolism (CYP7A1)
  - Insulin (HFD)
  - Glucagon (Fasting)
  - Adipogenesis in vivo

- **Pancreas**
  - Thermogenesis (UCP1)
  - Insulin (HFD)
  - Glucagon (Fasting)

- **BAT**
  - Thermogenesis (UCP1)

- **WAT**
  - Adipogenesis

**Clock**
- Circadian rhythm (BMAL1, CLOCK)

**Target genes**

Kojetin and Burris, Nat Rev Drug Discovery, 2014
A mechanistic glance at nuclear receptor superfamilly

Class II Nuclear Receptor

Co-repressor complex

Co-activator complex

Mediator complex

Ligand

Response Element

Target genes

Class II Nuclear Receptor

Pourcet B et al., Exp Opinion Emer Drugs, 2006
**Transcriptional activity of Rev-erbα**

**Natural ligand**
- Heme

**Synthetic ligands**
- GSK4112
- SR9009
- SR9011
- GSK2945

**Antagonists**
- SR8278

**Homodimer/Monomer**
- (Rev-erbα)₂
- AGGTCA(X)₂AGGTCA
- RevRE/RORE

**Tethering**
- NCoR
- Rev-erbα
- HNF6
- AGGTCA
- HRE

**Target gene**

**Rev-erbα is a transcriptional repressor**

NLRP3 inflammasome pathway displays circadian rhythmicity

CD11b+ Peritoneal Exudate Cells (PEC)

Peritoneal lavage

**Relative rev-erba mRNA levels**

**Relative nlrp3 mRNA levels**

**Relative il1β mRNA levels**

qPCR

ELISA

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α controls NLRP3 inflammasome daily oscillations

Peritoneal lavage

CD11b+ Peritoneal Exudate Cells (PEC)

Pourcet, Zecchin et al., Gastroenterology 2018
Circadian rhythmicity in primary macrophages

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α inhibits NLRP3 expression in primary macrophages

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erbα is recruited to the NLRP3 promoter in BMDM

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α inhibits NLRP3 activation in primary macrophages

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α inhibits caspase 1 maturation and IL-1β secretion in an NLRP3-dependent manner

Pourcet, Zecchin et al., Gastroenterology 2018
Pharmacological activation of Rev-erb-α modulates NLRP3 inflammasome pathway in vitro and in vivo

Pourcet, Zecchin et al., Gastroenterology 2018
Fulminant Hepatitis or Acute Liver Failure (ALF)

Drugs (50%)
- Acetaminophen (80%)
- Alcohol - Acetaminophen

Virus
- Hepatitis A, B, C

Toxic
- Amanite Phalloïde

Metabolism
- Wilson disease (ATP7B)
- Galactosemia
- Tyrosinemia

Tissue necrosis
Haemorrhage
Inflammation

200-300 cases per year
No treatment except liver transplant

Acute liver failure models

• **Surgical**
  - Anhepathic (hepatectomy)
  - Devascularized (Hepatic artery ligation)

• **Chemical model**
  - Acetaminophen (production of N-acetyl parabenzquinoneimine)
  - LPS/Galactosamine (inhibits hepatic transcription and promotes necrosis)
Death is a matter of time...

In CD11b⁺ peritoneal exudate cells

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erba deficiency increases fulminant hepatitis in an NLRP3-dependent manner.

Pourcet et al., Gastroenterology 2018
Rev-erb-α prevents fulminant hepatitis in a NLRP3-dependent manner

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α inhibits NLRP3 pathways in recruited hepatic macrophages

Liver F4/80+ cells

Relative Nlrp3 mRNA levels

DMSO
SR9009

Ctrl
LPS
GalN
LPS
GalN
MCC950

Liver F4/80+ cells

Relative Il1β mRNA levels

DMSO
SR9009

Ctrl
LPS
GalN
LPS
GalN
MCC950

Liver F4/80+ cells

Relative Il18 mRNA levels

DMSO
SR9009

Ctrl
LPS
GalN
LPS
GalN
MCC950

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α impairs neutrophils recruitment in an NLRP3-independent manner

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α prevents macrophages recruitment in an NLRP3-independent manner

Pourcet, Zecchin et al., Gastroenterology 2018
Rev-erb-α controls circadian rhythmicity of the NLRP3 inflammasome pathway

**Acute inflammation (peritonitis)**
- Rev-erb-α regulates IL-18 and IL-1β secretion in an NLRP3-dependent manner
- Rev-erb-α controls NLRP3-mediated circadian secretion of IL-1β and IL-18

**Chronic inflammation (atherosclerosis)**
- Rev-erb-α decreases atherosclerosis progression during ageing
- Rev-erb-α inhibits NLRP3 inflammasome pathway in aortic lesions
- Rev-erb-α inhibits vascular calcification
A role of the Rev-erb-α / NLRP3 axis in allergy?

The circadian clock and asthma

NLRP3 and asthma?

Durrington et al, BMJ, 2013

De Nardo et al, Am J Patho, 2014
Take Home Message

• Disruption of Clock = Disruption of many pathophysiological pathways

• DAMP/PAMP susceptibility depends on the time of day

• Chronotherapy
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