Novel pathway to control interferons and inflammation

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Type I IFN are powerful molecules: induce activation of 300-600 genes (called ISG)

IFNAR are expressed by virtually all the human cells
Type I IFN: the good, the bad, and the ugly

- Immune Surveillance
- Viral infection
- Bacterial infection
- Tumor
- Chronic viral and bacterial infections
- Depression
- Autoimmune diseases
- Interferono-pathies
  - SLE
The IFN producers: the plasmacytoid DCs

- Immune cells discovered in 1997, role during innate and adaptive immunity
- **Professional of IFNα** production (up to 1000x than other cell type)
- Powerful but very rare cells: less than 0.5% of PBMC
- Express innate immune sensors of the **Toll-like receptors** family (TLR-7 and -9)
- Express high levels of IRF-7 (regulating IFN genes) and the chemokine receptor **CXCR4**
- **pDC** represent promising cellular target for interferonopathies (SLE, sclerodermia..)

Sascha Rodes and Nikaïa Smith, Ulm University-CBMIT
Interferons and interferon producing cells

NCI, NIH USA (2001-2006)
This is the first demonstration that natural molecules could block pDCs
CNRS, Necker Hospital (2006-2012)

Modulation of interferon production
Histamine overproduction in atopic children increases viral infection

- Naturel Ligand of HR (GPCR)
- **Very unstable (Histaminase)**
- Endogenous monoamine
- $K_m$ (H1R et H2R) = 1000nM
- $K_m$ (H3R et H4R) = 5-10 nM

- Pharmacologic analogue
- Very stable
- Agoniste H4R – $K_m$ = 1nM
- Antagoniste H3R – $IC_{50}$ = 10nM
Effect of Histamine and CB on pDC activation

- Histamine
- Clobenpropit (CB)

pDC

IFN-I
ELISA
FACS

IFN-α production by HIV-stimulated pDC

- IFN-α production (ng/mL) - ELISA

Mock
Flu
Flu + HA
Flu + CB

- pDC
- Non Stimulated
- Flu
- Flu + CB (µM)

IFNa
Effect of Histamine/CB in vivo

Pathogenic potential of interferon αβ in acute influenza infection

Sophia Davidson, Stefania Crotta, Teresa M. McCabe & Andreas Wack

X31 very pathogenic Flu:

- ↑ IFNα, IFNβ in BAL
- ↑ Cytokines
- High activation of pDC
Effect of Histamine/CB in vivo

CB or Histamine 450µg/30µL/mouse 18h before infection

Influenza A virus X31 (H3N2)

Broncho-alveolar Wash 3 days post-infection
Elisa : IFNa, IFNb

S129S8 12 week mice

A. Wack (F. Crick Institute)

IFN-a

Protein production (pg/mL)

3000
2000
1000
0

Naive Veh Ctrl Histamine Cloprexipit

IFN-b

Protein production (pg/mL)

400
300
200
100
0

Naive Veh Ctrl Histamine Cloprexipit

Smith et al Nat Comm 2017
Identification of the receptor controlling interferon production
Chemistry & Biology, Modeling & Immunology for Therapy (CBMIT)

Understanding the regulation of type I interferons and inflammation

**Fundamental research:**
Production and regulation of IFN-I

- Cellular model: innate immune pDC, Monocytes
- Integrated model: Human tonsils
- *In silico* modeling
- Organic chemistry
- Molecular/cellular screening

**Translational research:**
Evaluation of therapeutic molecules

- Auto-immunity
- Interferonopathies
- Viral infection
- Selection of best molecules

**Clinical network**

*In silico docking* → *Functional screening* → *Organic chemistry* → *Optimization* → *In vitro* → *Ex vivo* → *In vivo* → *Patients*

Auto-immune pathologies
Interferonopathies
The chemokine receptor CXCR4 hypothesis

Hypothesis
Amine’s activity is due to:

Their receptor (HR, SR..) → No → Bind to Toll-like R → No → A common receptor

✓ Amino compounds bind to CXCR4 expressed by neurons
✓ CXCR4 is highly expressed by human pDC
✓ CXCR4 is a member of the GPCR family (as amine natural receptors)

Could CXCR4 be the common receptor?
The chemokine receptor CXCR4: pleiotropic activity

- Expressed by Hematopoietic Immune cells Neurons Tumor cells
- Extracellular domain
- Natural ligand: CXCL12 GPCR family member 7 transmembrane

Physiological
- Cell migration
- Chimiotaxism

Pathological
- HIV coreceptor
- Tumor migration

Could CXCR4 be the common receptor?
Silencing of CXCR4 in primary cells

Primary human pDC

CXCR4 siRNA

Histamine or CB
+ Flu

siCTL / siCXCR4

Relative mRNA expression levels

IFNα

Histamine
CB

siCTR
siCXCR4

Mock

Flu

Flu

Relative mRNA expression levels
CXCR4 specific ligands effect on type I IFN

CB is not a CXCR4 specific ligand, so what about CXCR4 ligands?

- **CXCL12**
  - Natural Ligand
  - Protein
  - Metastasis migration

- **AMD3100 Plerixafor**
  - Other name Plerixafor
  - Stable/clinically used
  - IC$_{50}$ CXCR4 = 5-10 nM
  - EC$_{50}$ antagonist: 10 nM

- **IT1t**
  - Synthetic ligand of CXCR4
  - Very stable
  - Developed by Novartis
  - IC$_{50}$ CXCR4 = 5-10 nM
  - EC$_{50}$ antagonist: 10 nM

Structural similarities

CB / IT1t
Immunomodulation of IT1t versus AMD3100

Inhibition of IFNa production by CXCR4 ligands

Purified pDC

μM AMD

R848 + Compound (µM)

μM IT1t

IFNα production (% of R848 stimulated pDC)

***,xx

**,xx

*,xxx

****,x

0

0.5

1

5

10

20

50

µM AMD

µM IT1t
Targeting CXCR4 \textit{ex vivo} with IT1t: SLE model
Systemic lupus erythematus (SLE) is defined by a complex clinical syndrome (e.g., arthritis, skin rashes, serositis, glomerulonephritis, nervous system involvement) and the production of antinuclear antibodies (ANAs).

Some individuals develop a type of skin disease, called cutaneous lupus erythematosus.
pDC & IFN: central players in lupus pathology

Kaul et al., (2016) Nat Rev Diseases Primer
Lupus is an interferonopathy

- Increased serum levels of IFN-α observed in many SLE patients correlate with both disease activity and key disease markers
  
  Guiducci *et al.*, Nature 2010

- Inhibition of IFN-α/β induced TRAIL expression may reduce symptoms in SLE by stopping pathogenesis of autoantibody production and autoimmune tissue injury
  
  Zahn *et al.*, British j. derm 2011

- Over-production of **Type I interferon (IFN-I)** is strongly associated with SLE and is involved in disease pathogenesis.
  
  *Rodero et al.*, JEM 2017
Ex vivo effect of IT1ton SLE patient

Brigitte Badder-Meunier
Pierre Quartier

Spl Juvenil Lupus Patients

PBMС

 +/- IT1t

overnight

➢ STAT activation
➢ IFNα production
➢ Simoa

➢ pSTAT1 (= IFN-I, II)

➢ pSTAT3 (= IL-6)

Darragh Duffy

Mathieu Rodero

Simoa

JSLE

JSLE

IFNα

Ex vivo effect of IT1ton SLE patient

Simoa: IFNα
Ex vivo effect of IT1t on SLE patient

Anne-Sophie Korganow

+ R848 +/- IT1t overnight

PBMC

IFNα production

Lupus patients

Ultrasonic digital IFNα ELISA (Simoa)

Intracellular staining of IFN-α

Ex vivo effect of IT1t on SLE patient

N. Bekadour
N. Smith

IFNα (fg/ml)

Unst 0 5 20

R848 + IT1t (µM)

HC3 HC4 HC5 HC6

P3 P4 P5 P6

SLE

0.34
0.29

0 1 10 20

HC2 P2

NS R848 R848 + IT1t

BDCA-4 SSC-A

IFNα

0.34
0.29

1 10 1
Targeting CXCR4 with IT1t \textit{in vivo}: Pristane-induced lupus in mice
Pristane SLE mouse model

Pristane-induced SLE in mouse model

Pristane (IP) daily
+/- Prednisolone
+/- IT1t 3 doses

Cytokine in serum
Anti-DNA antibodies at Week 4,8,10

10 Male DBA1/J

10 weeks
IT1t effect on cytokine production in SLE in mice

- SLE is characterized by over-production of cytokines: IL-1β, IL-6, IL-17 and TRAIL
- IL-17 Accurate Biomarkers for Systemic Lupus Erythematosus Disease Activity

![Graphs showing cytokine production over weeks 4 and 10 for IL-1β, IL-17, and TRAIL with different treatment groups: Vehicle, Prednisolone, IT1t - 3mpk, IT1t - 10mpk, IT1t - 30mpk.]
Systemic lupus erythematosus (SLE) is defined by a complex clinical syndrome and the production of antinuclear antibodies (ANAs).
The chemokine receptor CXCR4 (GPCR)

**Well-known activity**
- Cell migration
- Antagonist
- Inhibition cellular migration
- Anti-tumoral

**Novel activity**
- Immune modulation
- Biased agonist
- Inhibition of immune cells
- Auto-immune diseases
IFN-driven auto-immune diseases

Inflammation-driven auto-immune diseases

Drug design and optimization
Heat/leads development
Preclinical studies
Clinical studies

IFN-driven auto-immune diseases

Inflammation-driven auto-immune diseases
Summary and highlights of CBMIT (2013-2019)

- **Publications**
  Total: 86
  (Nat Com, Science Adv, Immunity, Plos patho..)
  Interface Immunology Virology
  Neurology Oncology Chemistry

- **Valorization**
  6 patents (SATT)
  4 maturation contracts (SATT)
  Creation of SantImmune (POV-JPH)

- **Start-Up**
  Ermium Therapeutics

- **Collaborations**

- **Grants**

- **Multimedia**
  - TV (2)
  - Radio (1)
  - Press (12)

- **Valorization**
  6 patents (SATT)
  4 maturation contracts (SATT)
  Creation of SantImmune (POV-JPH)

- **Collaborations**

- **Expertise**

- **Multimedia**
  - TV (2)
  - Radio (1)
  - Press (12)

- **Cover**

- **Valorization**
  6 patents (SATT)
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  Creation of SantImmune (POV-JPH)