Even more receptor families

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JAK/STAT Cytokine Receptors
Discovery of interferons

Virus interference. I. The interferon

By A. Isaacs and J. Lindenmann*

National Institute for Medical Research, London

(Communicated by C. H. Andrewes, F.R.S.—Received 7 March 1957)

During a study of the interference produced by heat-inactivated influenza virus with the growth of live virus in fragments of chick chorio-allantoic membrane it was found that following incubation of heated virus with membrane a new factor was released. This factor, recognized by its ability to induce interference in fresh pieces of chorio-allantoic membrane, was called interferon. Following a lag phase interferon was first detected in the membranes after 3 h incubation and thereafter it was released into the surrounding fluid.

IFN-induced gene expression

Figure 3. Induction of mRNAs by Different Amounts of IFN

Cells were treated for 8 hr with the indicated concentrations of IFN. Total RNA (200 μg/slot) was bound to nitrocellulose and probed with each cDNA. The experiments shown in A and B were carried out on different days. As noted in the text, levels of the mRNAs in untreated cells varied from day to day.
<table>
<thead>
<tr>
<th>Year</th>
<th>Milestone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1957</td>
<td>Isaacs and Lindenmann describe interferon</td>
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<tr>
<td>1975–1977</td>
<td>Oligonucleotide [2′-5′] oligoadenylates(s)] inhibitors of protein synthesis induced by IFN found</td>
</tr>
<tr>
<td>1979</td>
<td>Actinomycin-sensitive IFN-β-dependent new protein induction shown</td>
</tr>
<tr>
<td>1984</td>
<td>IFN-α-induced transcriptional stimulation of specific genes (ISGs) demonstrated; no new protein synthesis required</td>
</tr>
<tr>
<td>1986–1988</td>
<td>IFN-dependent promoters identified (ISREs, interferon-stimulated response elements)</td>
</tr>
<tr>
<td>1988–1989</td>
<td>IFN-α-induced ISRE binding protein complexes (ISGF3; E complex) in cytoplasm in 1–2 min; in nucleus in 5 min</td>
</tr>
<tr>
<td>1989</td>
<td>Genetic selection system for defective IFN-induced transcription described and first cell mutant selected</td>
</tr>
<tr>
<td></td>
<td>IFN-γ-dependent promoters (GAS, gamma IFN-activated sequences, and GAF, gamma IFN-activated factor) identified</td>
</tr>
<tr>
<td>1989–1991</td>
<td>JAK1 and JAK2 and TYK2 identified</td>
</tr>
<tr>
<td>1990</td>
<td>ISGF-3 partially purified; identified subunits 113, 91, 84, 48</td>
</tr>
<tr>
<td>1991</td>
<td>Noncomplementing mutant cells unresponsive to both IFN-α and IFN-γ described</td>
</tr>
<tr>
<td>1992</td>
<td>cDNA clones sequenced later called STAT1 (a and b) and STAT2; RNA for IRF9 completing make up of ISGF3, establishing STAT family of proteins</td>
</tr>
<tr>
<td></td>
<td>First IFN response mutant identified as Tyk2 by molecular complementation</td>
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<tr>
<td></td>
<td>Upon IFN activation by IFN-α STAT1 and STAT2 are tyrosine phosphorylated; STAT1 also tyrosine phosphorylated after IFN-γ treatment</td>
</tr>
<tr>
<td>1993–1994</td>
<td>Major signaling events driven by IFN and IL-6 pinpointed by molecular complementation of mutant cells</td>
</tr>
<tr>
<td>1994</td>
<td>JAK3 described and sequenced</td>
</tr>
<tr>
<td>1994–1995</td>
<td>STAT3, 4, 5A, 5B, and 6 all described and sequenced</td>
</tr>
<tr>
<td>1995–1998</td>
<td>Functional and structural domains of STATs described</td>
</tr>
<tr>
<td>1996</td>
<td>Drosophila STAT (dSTAT92E) first described; later studied extensively genetically</td>
</tr>
<tr>
<td></td>
<td>Mouse genetics identifies physiological functions for all STATs in various specific cells</td>
</tr>
<tr>
<td>1997</td>
<td>Negative regulation of pathways initially characterized</td>
</tr>
<tr>
<td>1998</td>
<td>First crystal structures of STATs</td>
</tr>
<tr>
<td>2000</td>
<td>Initial information that human mutations in JAKs and STATs and persistent activation of STATs cause disease</td>
</tr>
<tr>
<td></td>
<td>First posttranslational modifications of STATs in addition to phosphorylations noted (methylation, acetylation, etc.)</td>
</tr>
<tr>
<td>2001</td>
<td>Comprehensive gene target sets identified</td>
</tr>
</tbody>
</table>
Class I and II Cytokines
Components for response to cytokine:
- Receptor
- Receptor Kinase (JAK)
- Transcription Factor (STAT)
- SOCS – suppressor of cytokine signaling
CHR – cytokine receptor homology region – composed of FnIII domains

Cytokines bind at the junction between two FnIII domains
The molecular details of cytokine signaling via the JAK/STAT pathway
• Four members of JAK family: JAK1-3 and Tyk2

• FERM and SH2 domains tether JAK to the receptor
  • FERM binds Box I
  • SH2 binds Box II (through a Glu, not a pTyr)

• Pseudokinase (ψK) regulates kinase activity
  • V617F mutation of JAK2 results in a hyperactive kinase associated with myeloproliferative diseases

• JAKs phosphorylate activation loops in trans like RTKs
• Activated JAK phosphorylates the receptor, promoting STAT binding through the SH2 domain

• JAKs also phosphorylate Tyr residue between the SH2 and transactivation domain
  • SH2 domain then binds to the pTyr of the partner in the dimer
Negative regulation of cytokine receptor signaling

SOCS proteins can inhibit signaling by: 1) binding the receptor and promoting its degradation by ubiquitination; and 2) directly inhibiting JAKs (for SOCS1 and SOCS3)
Myelofibrosis – scarring of the bone marrow

Normal

Myelofibrosis

https://imagebank.hematology.org/
JAK2 and Growth Factor Receptor Mutations in Myelofibrosis

*Cancer Chemotherapy and Pharmacology* volume 77, pages 1125–1142 (2016)
Treatment of Myelofibrosis with a JAK1/2 inhibitor: Ruxolitinib

TNF-α receptor superfamily
TNFs

• 1968: Gale Granger and Nancy Ruddle – Cytotoxic factor produced by lymphocytes (lymphotoxin)

• 1975: Lloyd Old – Cytotoxic factor produced by macrophages and named it tumor necrosis factor (TNF). Factors could kill fibrosarcoma cells.

• 1984: cDNAs encoding LT and TNF were cloned in 1984 – similar structure

• 1985: Bruce Beutler and Anthony Cerami – cachectin was actually TNF
Wasting disease (cachexia) in a cow with African trypanosomiasis
Cachectin = Mouse tumor necrosis factor

(mouse CACH)

H₂N LEU-ARG-SER-SER-GLU-ASN-SER-SER-ASP-PRO-PRO-VAL-ALA-? -VAL-VAL-ALA-ASN...


(human TNF)

1 µg of cachectin had 10^2 U of TNF activity
This raised the question: might TNF mediate all effects of LPS, including the lethal effect?

- Triglyceride synthesis, LPL, FAS
- AcCoA carboxylase, glycerol release

LPS → MACROPHAGE → TNF → TUMOR

- Tumor necrosis and cytolysis
Purified TNF mimics LPS toxicity

1984

Denatured TNF
Active TNF
Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey

1984

1985

1986

1989

1990

1991

1992

1994

1995

1996

1997

1998

1999

2000

2001

2003

2004

2011

LT (TNF-β)

Ligands

TNF-α

LT-β

CD40L

4-1BBL

CD27L

TRAIL

LIGHT

RANKL

TWEAK

APRIL

BAFF

VEGI

EDA-A1

EDA-A2

GITRL

Receptors

1984

TNFR1

CD30

4-1BB

TNFR2

Fas

LT-βR

OX-40

CD27

CD40

DR5

DR3

DR4

DcR1

RANK

LIGHT

GITR

DR6

OPG

DcR2

DcR3

EDAR

XEDAR

TROY

TACI

RELT

BAFFR

Adaptors

1984

TRAF1

TRAF2

TRAF3

TRAF4

TRAF5

TRAF6

FLICE

RIP

FADD

EDARADD

Act1

Bharat B. Aggarwal, Subash C. Gupta, Ji Hye Kim, Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey, Blood, 2012, Figure 2
TNF Receptor Family

Cross-linking of TNF-R1 by TNF

TNF-R1

"Death domain"

TNF

Binding of adaptor protein (TRADD)

Binding of signaling intermediates (TRAF, RIP, FADD)

Generation of active transcription factors (AP-1, NF-κB)

MAP kinase cascade

Active JNK

Active AP-1

Gene transcription: production of inflammatory mediators, survival proteins

 Activation of effector caspases

Active caspase-8

IκB kinase cascade

IκB

NF-κB

IκB

Apoptosis
TNF receptor superfamily

http://dx.doi.org/10.1016/B978-0-12-407707-2.00005-9
Downstream activation of TRAFs and TAK1

TRAF2 and 6 structure

RING domains dimerize while the coiled coil trimerizes

Activating receptor interaction with TRAF2

Linking death domains to TRAF2

TAB2 interacting with K63-linked di-Ub complex

http://dx.doi.org/10.1016/B978-0-12-407707-2.00005-9
Death-induced signaling complex

Catalytic domain structure of caspase-8

http://dx.doi.org/10.1016/B978-0-12-407707-2.00005-9
RIP1/3 programmed necrosis - “necroptosis”
Signaling networks in immunity
Anti–Tumor Necrosis Factor α (TNF-α) Antibodies in Inflammatory Bowel Disease.
Rates of Clinical Response and Remission after a Single Infusion of cA2 or Placebo.

![Graph showing response and remission rates over weeks for different groups with statistical significance notes (P<0.001 for response, P=0.005 for remission).]

**NO. OF PATIENTS EVALUATED**

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>25</td>
<td>24</td>
<td>27</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>5 mg of cA2/kg</td>
<td>27</td>
<td>26</td>
<td>23</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>10 mg of cA2/kg</td>
<td>28</td>
<td>24</td>
<td>27</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>20 mg of cA2/kg</td>
<td>28</td>
<td>27</td>
<td>27</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>All cA2 groups</td>
<td>83</td>
<td>77</td>
<td>80</td>
<td>83</td>
<td>83</td>
</tr>
</tbody>
</table>

Pattern recognition receptors

Toll-like receptors
NOD-like receptors
RIG-I-like receptors
Gram negative bacteria

*Eschericia coli*
Biosynthesis of Lipid A

Christian R.H. Raetz
1946 - 2011

LpxA
LpxC
LpxD
LpxH
LpxB
LpxK

UDP-GlcNAc
UDP-Diacyl-GlcN
Disaccharide-1-P

Kdo2-Lipid A
C14-ACP
Kdo2-IVα

Lipid IVα

Bruce Beutler – Nobel Lecture
Discover of TLRs

A human homologue of the Drosophila Toll protein signals activation of adaptive immunity

Ruslan Medzhitov, Paula Preston-Hurlburt & Charles A. Janeway Jr

Section of Immunobiology, Yale University School of Medicine, and *Howard Hughes Medical Institute, New Haven, Connecticut 06520-8011, USA

NATURE | VOL. 388 | 24 JULY 1997

- IFNγ
+ IFNγ

THP-1 T12 T17 THP-1 T12 T17

actin
IL-1
IL-8
IL-6
B7.1

Fold induction
Structure of Leucine-Rich Repeats in the ECD

A  
TLR  XLXXLXLXXNXLXXXLXXXXFXXLX
RI  XLXXLXLXXNXLXXXXLXXXXLXXXX

B  

C  

TLR3  
RI  

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Ligand-binding promotes dimerization
Figure 4

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TLR3/dsRNA complex
Toll IL-1 receptor (TIR) domains heterodimerize with effector TIR domains
## Development of TLR agonists as drugs

<table>
<thead>
<tr>
<th>Application of TLR Immunomodulator</th>
<th>Examples</th>
<th>Mechanism</th>
<th>Potential Therapeutic Outcome</th>
<th>Potential Adverse Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to infection</td>
<td>TLR2 agonist Pam2Cys, TLR4 agonists MPLA &amp; PHAD, TLR3 agonist poly I:C, TLR9 agonist CpG</td>
<td>Increased leukocyte recruitment and antimicrobial functions</td>
<td>Improved survival; reduced risk of nosocomial infections; reduced reliance on antibiotics</td>
<td>Chronic inflammation; autoimmune disease</td>
</tr>
<tr>
<td>Vaccine adjuvant</td>
<td>TLR4 agonist MPLA as an approved adjuvant in malaria (AS01), human papillomavirus (HPV), and hepatitis B (AS04) vaccines</td>
<td>Immune stimulation for increased antibody titers</td>
<td>Improved efficacy of vaccines and reduced dosing strategies</td>
<td>Discomfort at injection site; transient malaise</td>
</tr>
<tr>
<td>Cancer immunotherapy</td>
<td>TLR3 agonist poly I:C &amp; derivatives; TLR7 agonist 1V270</td>
<td>T-cell activation and DC maturation</td>
<td>Antitumor immunity</td>
<td>Dose-limiting side effects (fatigue, malaise, fever)</td>
</tr>
<tr>
<td>Chronic infections &amp; inflammatory diseases</td>
<td>TLR4 antagonist Eritoran to treat sepsis; TLR9 agonist Lefftolimod for reduction of HIV-1 viral reservoir</td>
<td>Antagonize TLR to prevent activation and downstream inflammation</td>
<td>Reduced inflammation and associated organ injury</td>
<td>Immune tolerance</td>
</tr>
</tbody>
</table>

Nuclear receptor superfamily

Steroid hormone receptors

Thyroid receptor
Dr. Charles Huggins

- Huggins and Hodges first treated men with prostate cancer with either orchiectomy or estrogen
- Huggins and Bergenstal used adrenalectomy for the “immediate and persistent relief of crippling bone pain”

Nobel Prize 1966 for “his discoveries concerning hormonal treatment of prostatic cancer”

References:
Studies on Prostatic Cancer

I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate*

Charles Huggins, M.D., and Clarence V. Hodges, M.D.

(From the Department of Surgery, the University of Chicago, Chicago, Illinois)
(Received for publication March 22, 1941)


Nobel Prize 1966
for “his discoveries concerning hormonal treatment of prostatic cancer”
Discovery of the estrogen receptor

- Steroids were thought to act via enzyme activation, etc.
- 1950s (Jensen): radioactive estrogen to ovariectomized rats – localized to reproductive tissues. ER hypothesized.
- 1960s: O’Malley suggested nuclear concept based on mRNA induction by estrogen
- 1985 – Ron Evans cloned the glucocorticoid receptor

https://doi.org/10.1073/pnas.1301566110
Nuclear receptor physiology
Structure of the ERE-DNA/ERα/SRC-3/p300 Complex

*Molecular Cell* 57, 1047–1058, March 19, 2015
Activating mutations in the ERα ligand-binding domain

<table>
<thead>
<tr>
<th>Zone</th>
<th>Mutation site</th>
<th>Pharmacological phenotype</th>
<th>Likely mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. h11–h12 loop, near the amino terminus of h12</td>
<td>Y537S, Y537N, Y537C and Y537D</td>
<td>Strong constitutive activity and AE resistance Y537S &gt; Y537N ~ Y537C</td>
<td>Strong hydrogen bond to D351 stabilizes AF2 conformation and allows loop to pack better</td>
</tr>
<tr>
<td></td>
<td>D538G</td>
<td>Moderate constitutive activity and more easily reversed by AEs</td>
<td>Flexibility in linkage to h12 allows loop to pack better</td>
</tr>
<tr>
<td></td>
<td>L536R, L536H, L536P and L536Q</td>
<td>Modest constitutive activity but harder to reverse with AEs</td>
<td>Replacing leucine with hydrophilic residue eliminates hydrophobic penalty from water exposure</td>
</tr>
<tr>
<td>2. h5, near the carboxyl terminus of h12</td>
<td>E380Q</td>
<td>Weak constitutive activity and easily reversed by AEs</td>
<td>Relieves coulombic repulsion with two acidic residues in h12</td>
</tr>
<tr>
<td>3. h9–h10 loop</td>
<td>S463P</td>
<td>Moderate constitutive activity and easily reversed by AEs</td>
<td>Potentially stabilizes the dimer interface and/or a flexible loop and possibly enables further intra-domain interactions</td>
</tr>
</tbody>
</table>
If we have time

How peppers and peppermint identified sensory receptors for temperature and pain

David Julius
Department of Physiology
UC San Francisco