BIO-479: Exercise 1

Revision of Lecture 1

Immune activation cascade

What are the layers of defence that the innate immune system provides?

Innate immune system

e.g. epithelia lining internal and external surfaces

e.g. phagocytes beneach epithelial layer, antimicrobial peptide repertoire

recognition of pathogen-associated patterns through PRRs

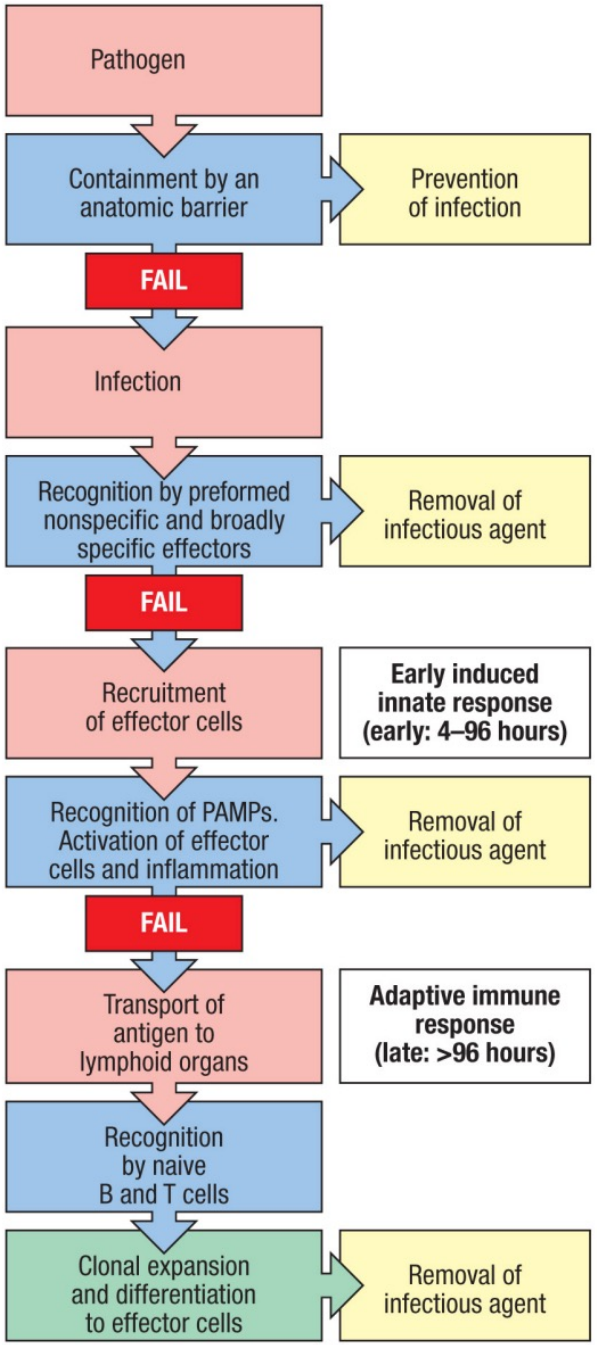


Figure 2.1 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

ANATOMICAL BARRIERS

PRE-FORMED EFFECTORS

RECRUITMENT OF EFFECTOR CELLS

BRIDGE TO ADAPTIVE IMMUNE SYSTEM

How would you define a pathogen?

Pathogen versus microbe

Definition of a **pathogen:** a microorganism that *makes us sick*

➔ has evolved strategies to overcome the body's defense system and thereby cause disease.

Definition of a pathogen linked to the status of an individual's immune system

➔ from the perspective of an individual with a reduced immune system:
more microorganisms can be dangerous
= « *opportunistic* » pathogens

Pathogen-induced damage

What are the direct and indirect mechanisms by which pathogens can exert damage to the host?

Means to cause disease

- *Exotoxin production*: secreted toxins from pathogens
- *Endotoxin production*: tissue damage by non-secreted constituents of pathogens - LPS - via the host
- *Direct cytopathic effect*
- Indirectly via *immune-mediated tissue damage*

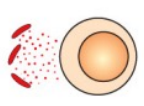
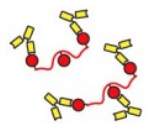
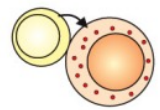
	Direct mechanisms of tissue damage by pathogens			Indirect mechanisms of tissue damage by pathogens		
	Exotoxin production	Endotoxin	Direct cytopathic effect	Immune complexes	Anti-host antibody	Cell-mediated immunity
Pathogenic mechanism						
Infectious agent	*Streptococcus pyogenes* *Staphylococcus aureus* *Corynebacterium diphtheriae* *Clostridium tetani* *Vibrio cholerae*	*Escherichia coli* *Haemophilus influenzae* *Salmonella typhi* *Shigella* *Pseudomonas aeruginosa* *Yersinia pestis*	Variola Varicella-zoster Hepatitis B virus Polio virus Measles virus Influenza virus Herpes simplex virus Human herpes virus 8 (HHV8)	Hepatitis B virus Malaria *Streptococcus pyogenes* *Treponema pallidum* Most acute infections	*Streptococcus pyogenes* *Mycoplasma pneumoniae*	Lymphocytic choriomeningitis virus Herpes simplex virus *Mycobacterium tuberculosis* *Mycobacterium leprae* *Borrelia burgdorferi* *Schistosoma mansoni*
Disease	Tonsillitis, scarlet fever Boils, toxic shock syndrome, food poisoning Diphtheria Tetanus Cholera	Gram-negative sepsis Meningitis, pneumonia Typhoid fever Bacillarydysentery Wound infection Plague	Smallpox Chickenpox, shingles Hepatitis Poliomyelitis Measles, subacute sclerosing panencephalitis Influenza Cold sores Kaposi's sarcoma	Kidney disease Vascular deposits Glomerulonephritis Kidney damage in secondary syphilis Transient renal deposits	Rheumatic fever Hemolytic anemia	Aseptic meningitis Herpes stromal keratitis Tuberculosis Tuberculoid leprosy Lyme arthritis Schistosomiasis

Figure 2.4 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

The epithelium

How do our epithelial surfaces protect us from immune threats?

Mechanical / physical defences

- Tight junctions between epithelial cells prevent microorganisms from entering
- Mucus production can prevent the adhesion of pathogens to epithelial surfaces
- Peristalsis (smooth muscle contraction) in the gut helps expel microbes

Chemical defenses

- Enzymes e.g. in tears, saliva and the gut, low pH in the gut, fatty acids in the skin have antimicrobial functions

Microbiological defenses

- Commensal bacteria, such as lactobacilli, produce antimicrobial substances

Antimicrobial peptides (AMPs)

How do AMPs exert host protection?

What is an example of a cell type that stores AMPs?

Antimicrobial peptides

Antimicrobial peptides: huge class of evolutionary highly conserved substances (conserved in plants, drosophila), classified in 3 major types ➔ *defensins, cathelicidins, histatins*

e.g. neutrophils store α-defensins

- Short cationic peptides, which derive from an inactive propeptide through being processed by cellular proteases
- Common *amphipathic* structure (two separate regions: one positively charged, the other hydrophobic) ➔ functionally important!
- Act within minutes to disrupt the membrane of bacteria, fungi and also certain viruses involving the formation of a *membrane pore*

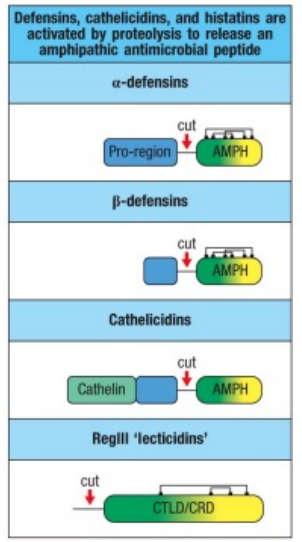


Figure 2.11 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

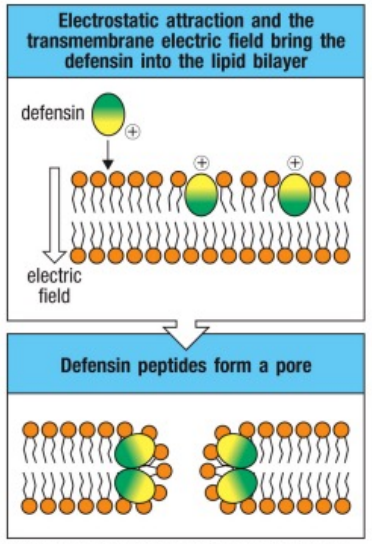


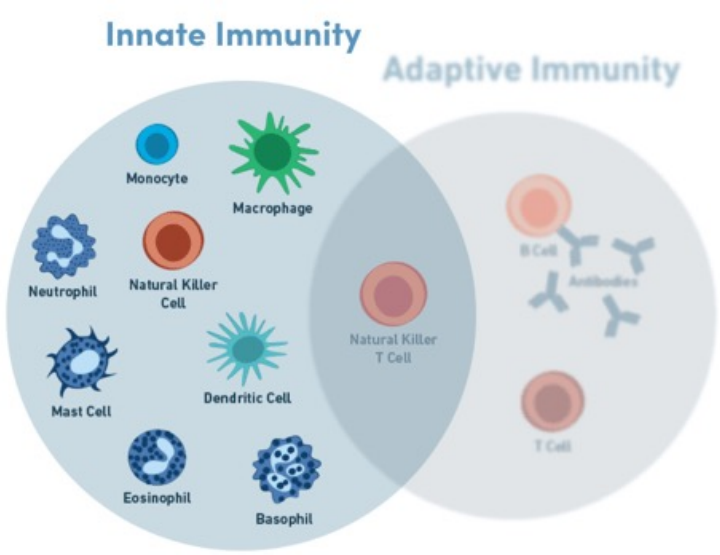
Figure 2.10 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

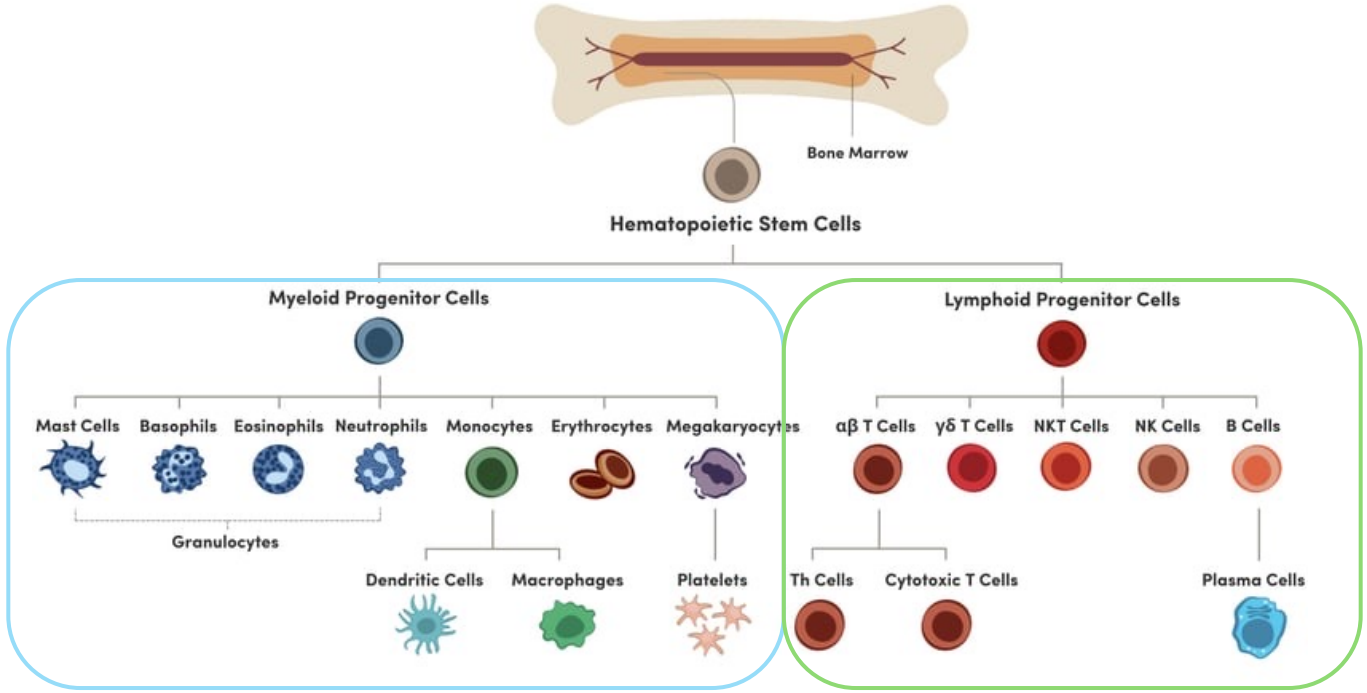
Overview of innate immune cell types

Which immune cell types can you list?

What are their different functions?

Overview of innate immune cell types



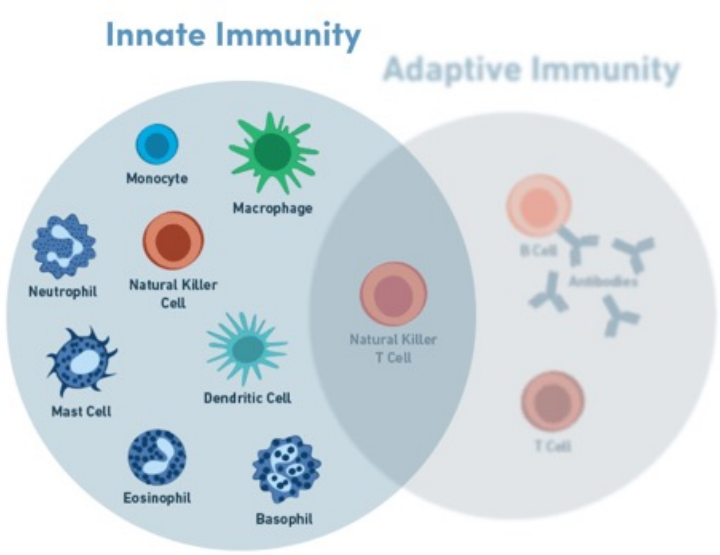


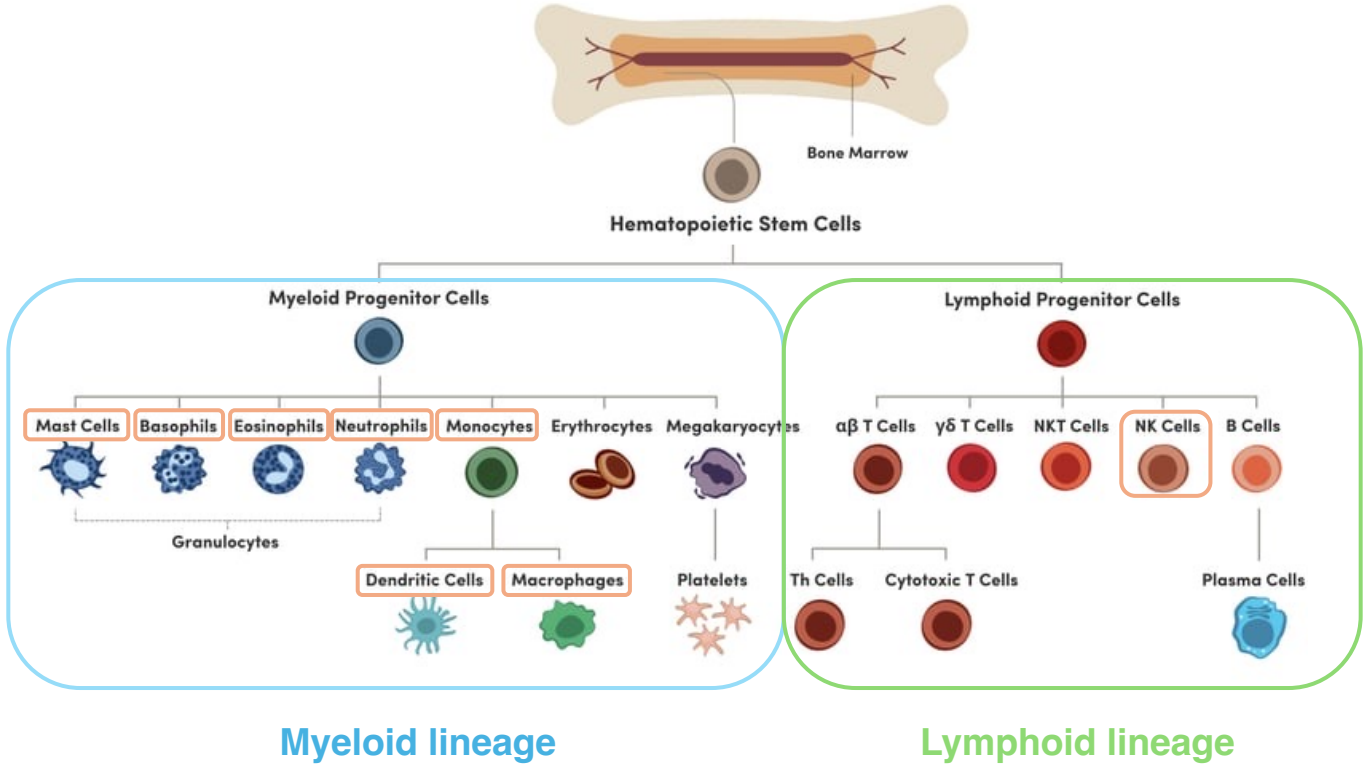
Myeloid lineage

Lymphoid lineage

Source: CST

Overview of innate immune cell types





Source: CST

Neutrophils

Which of the following is **not true** about neutrophils?

- Neutrophils are produced in the bone marrow
- Neutrophils can degranulate to release antimicrobial editors
- Neutrophils are long-lived cells
- Neutrophils are phagocytically active
- Neutrophils participate in the acute phase of inflammation

Neutrophils

Which of the following is **not true** about neutrophils?

- Neutrophils are produced in the bone marrow
- Neutrophils can degranulate to release antimicrobial editors
- **Neutrophils are long-lived cells**
- Neutrophils are phagocytically active
- Neutrophils participate in the acute phase of inflammation

Half-life in circulation: 6-12h, tissue lifespan only up to a few days

Neutrophils

- Neutrophils are produced in the bone marrow
- Neutrophils can degranulate to release antimicrobial editors
- Neutrophils are long-lived cells
- Neutrophils are phagocytically active
- Neutrophils participate in the acute phase of inflammation

They are both a type of granulocyte and a type of phagocyte !

Mononuclear phagocytes

Which cell types are mononuclear phagocytes?

Where are they localized?

What do they do?

Mononuclear phagocytes

- Includes monocytes – can migrate to tissues and become macrophages
- Includes tissue-resident macrophages
- Both are phagocytic
- Monocytes are circulating and can be recruited to infection sites
- Macrophages also dispose of apoptotic cells
- Macrophages also take care of tissue repair after injury

Mast cells

How do mast cells act in our immune system?

Mast cells

- Mast cells are another type of granulocyte
- They contain granules with inflammatory mediators such as histamine
- They are present in the skin and mucosal epithelia

Dendritic cells

List important physiological functions of dendritic cells

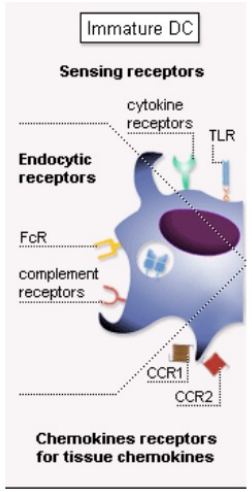
Dendritic cells

- Potent antigen-presenting cells (APCs)
- They can induce T cell response against antigens
- Immature DCs occur across tissues; they have high capacity for antigen uptake but limited antigen presentation capacity
- Mature DCs present in T cell areas; reduced antigen capture capacity, but high propensity for antigen presentation & T cell activation

DCs exist in two different states: immature and mature

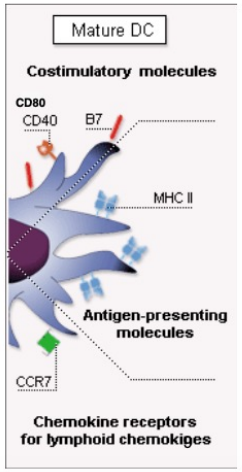
Immature DCs occur in all tissues where antigen may enter into the body

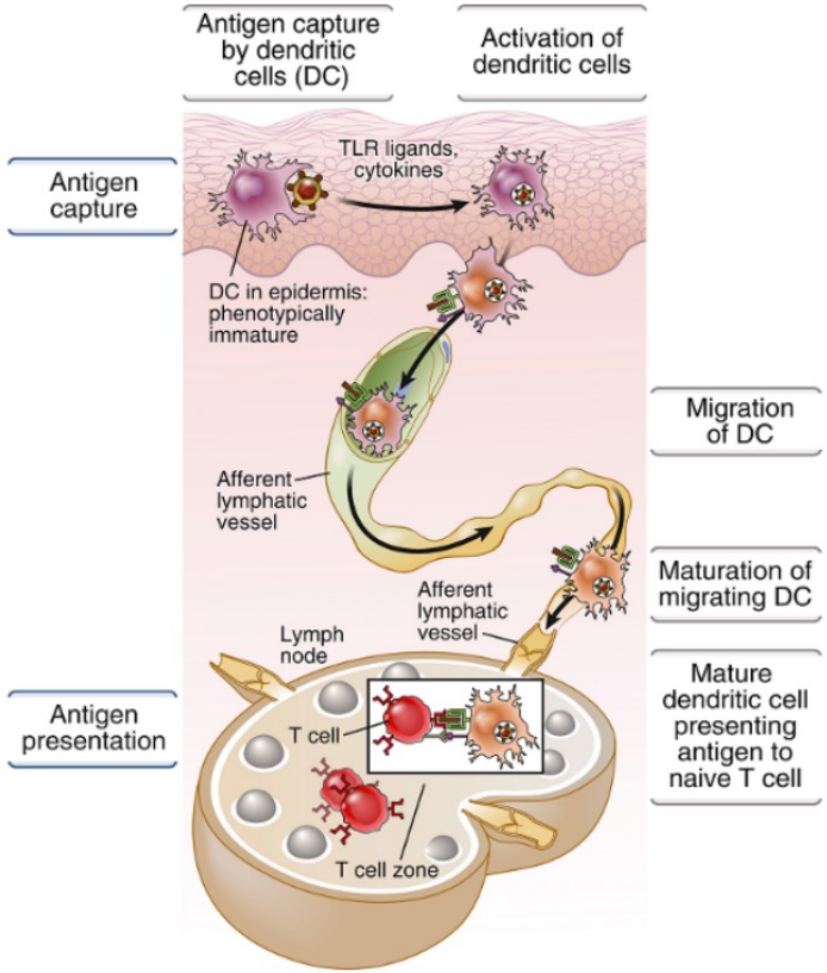
high capacity for antigen uptake but a limited capacity for antigen presentation



Mature DCs are exclusively found in the T cell areas of secondary lymphoid organs

They are characterized by a high propensity for antigen presentation and T cell activation but a reduced ability to capture antigens.



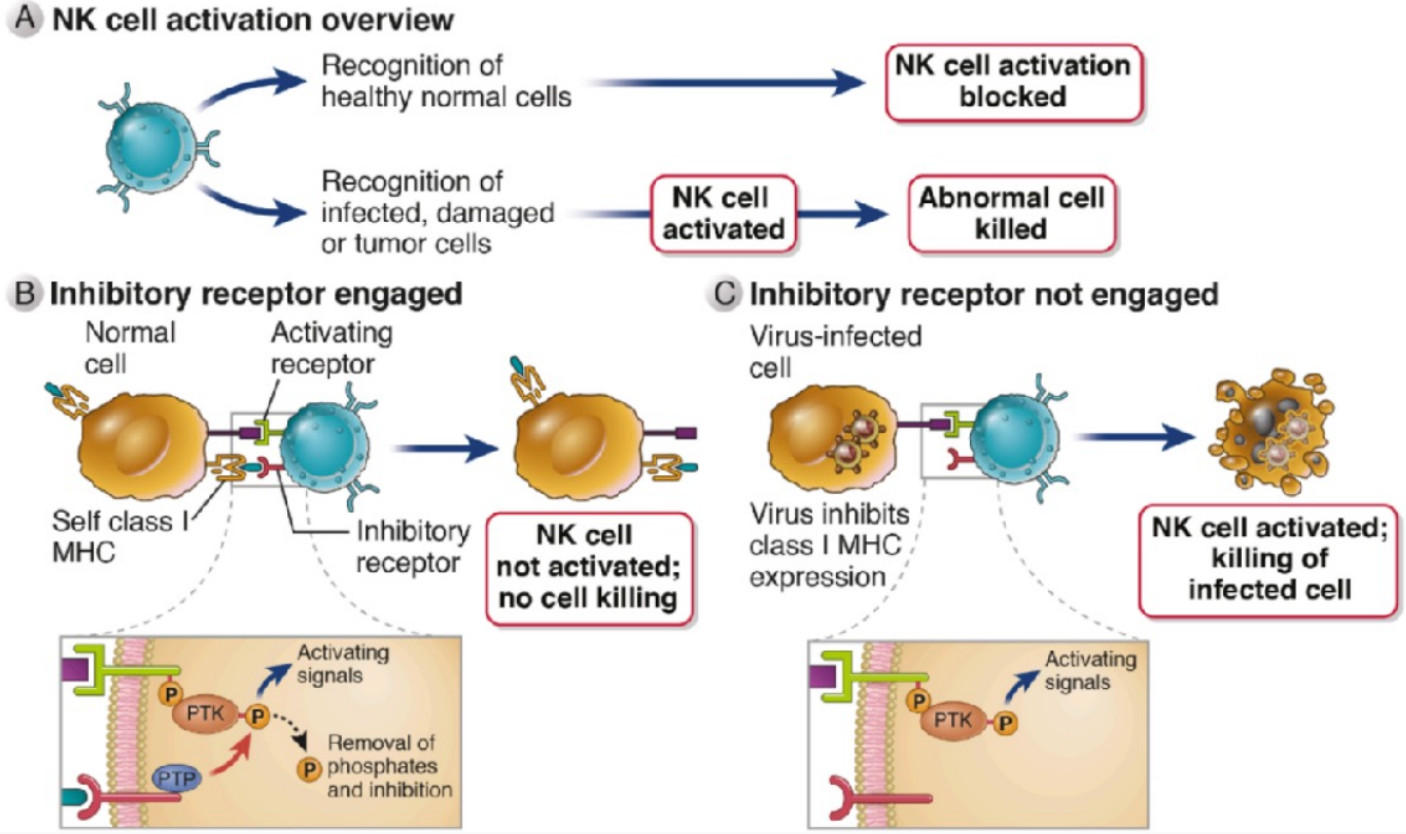


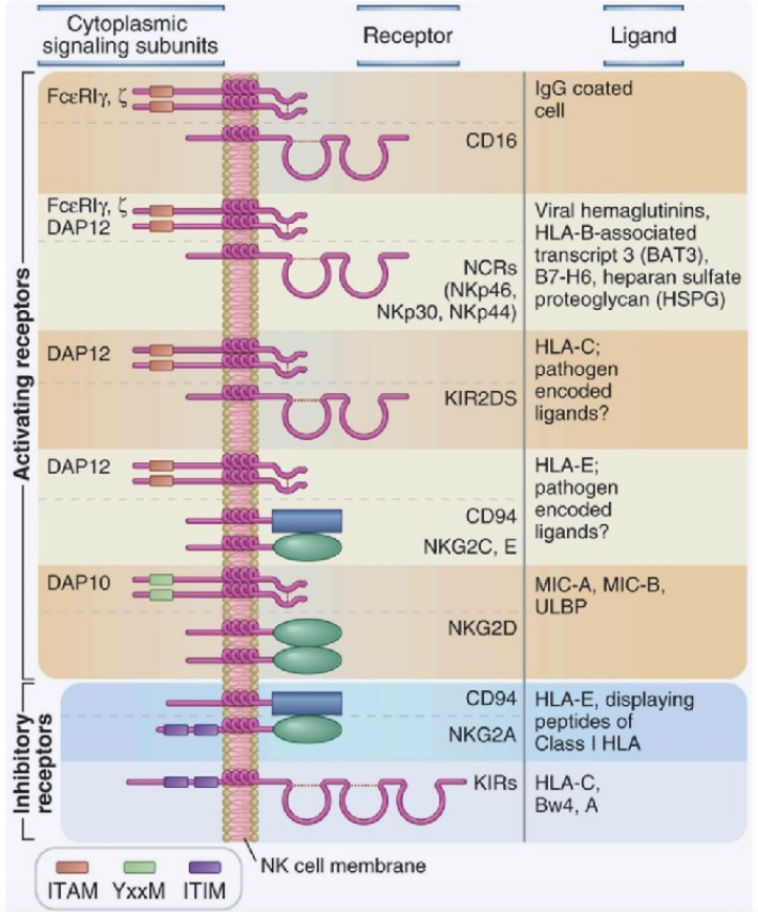
NK cells

How do NK cells recognize and kill target cells?

NK cells

- Contain cytotoxic proteins like granzyme or perforin
- Type I IFNs and other cytokines active their killing behaviour
- They distinguish healthy from infected cells through a combination of inhibitory and activating receptors


A NK cell activation overview
Recognition of healthy normal cells
NK cell activation blocked
Recognition of infected, damaged or tumor cells
NK cell activated
Abnormal cell killed
B Inhibitory receptor engaged
Normal cell
Activating receptor
Self class I MHC
Inhibitory receptor
NK cell not activated; no cell killing
Activating signals
PTK
PTP
Removal of phosphates and inhibition
C Inhibitory receptor not engaged
Virus-infected cell
Virus inhibits class I MHC expression
NK cell activated; killing of infected cell
Activating signals
PTK


Cytoplasmic signaling subunits
Receptor
Ligand
Activating receptors
FcεRIγ, ζ
CD16
IgG coated cell
FcεRIγ, ζ DAP12
NCRs (NKp46, NKp30, NKp44)
Viral hemaglutinins, HLA-B-associated transcript 3 (BAT3), B7-H6, heparan sulfate proteoglycan (HSPG)
DAP12
KIR2DS
HLA-C; pathogen encoded ligands?
DAP12
CD94 NKG2C, E
HLA-E; pathogen encoded ligands?
DAP10
NKG2D
MIC-A, MIC-B, ULBP
Inhibitory receptors
CD94 NKG2A
HLA-E, displaying peptides of Class I HLA
KIRs
HLA-C, Bw4, A
NK cell membrane
ITAM YxxM ITIM

Recognising immune threats

- NK cells detect microbes by integrating signals from inhibitory vs. activating receptors
- **But how do other cells decide when to eat (neutrophils / macrophages), when to activate T cells (dendritic cells), and when to produce antimicrobial peptides (epithelial cells)?**

Pattern recognition receptors (PRRs)

What is a pattern recognition receptors?

Give examples of receptor:ligand pairs!

Pattern recognition receptors (PRRs)

Bacteria, viruses, fungi can produce…

- Microbe-associated molecular patterns (MAMPs)
- Pathogen-associated molecular patterns (PAMPs)
- Damage-associated molecular patterns (DAMPs)

We have specialized receptors that can recognize these patterns and trigger different signaling cascades as a consequence

Examples of such PAMPs include nucleic acids (DNA, RNA), bacterial cell wall lipids, or carbohydrates

		Microbe Type
Pathogen-Associated Molecular Patterns		
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	—
Crystals	Monosodium urate	—
Proteolytically cleaved extracellular matrix	Proteoglycan peptides	—
Mitochondria and mitochondrial components	Formylated peptides and ATP	—
Nuclear proteins	HMGB1, histones	—

ATP, Adenosine triphosphate; *CpG*, cytosine-guanine–rich oligonucleotide; *dsRNA*, double-stranded RNA; *HMGB1*, high-mobility group box 1; *HSP*, heat shock protein; *LPS*, lipopolysaccharide; *ssRNA*, single-stranded RNA.

Why would DNA be an indicator of pathogen invasion?

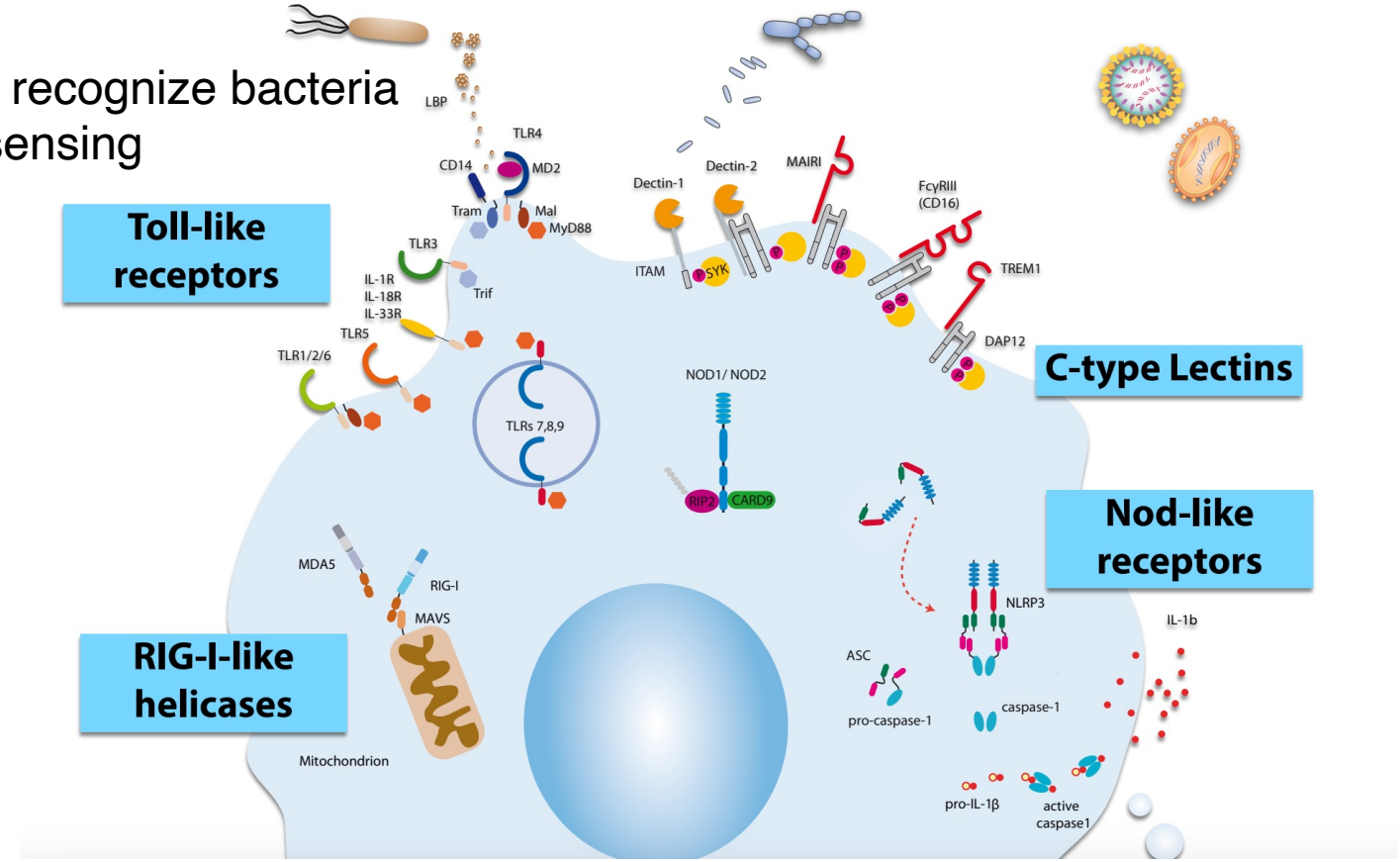
Overview of some key PRRs and their ligands

TLR3,7/8 can recognize RNA viruses

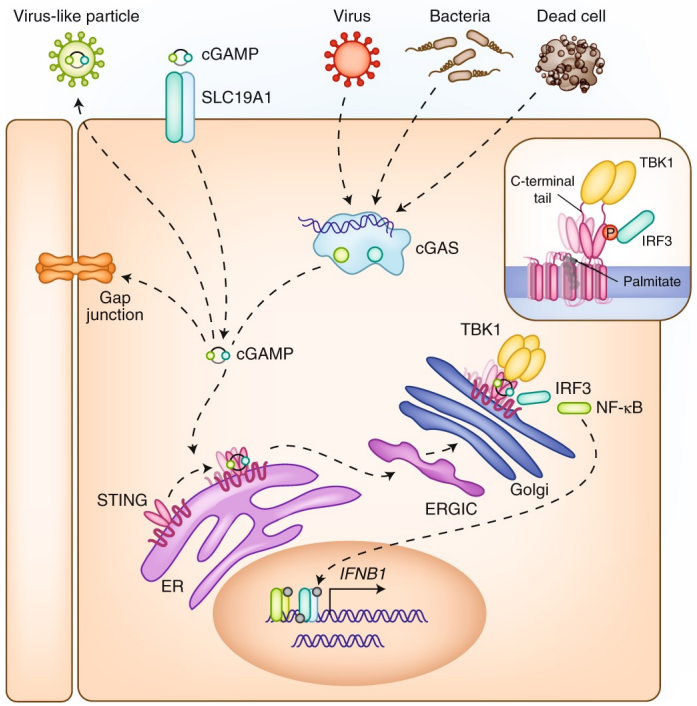
TLR9 can recognize DNA viruses

TLR2/4/5 can recognize bacteria e.g. via LPS sensing

C-type lectins such as Dectin-1 detect fungi-derived carbohydrates

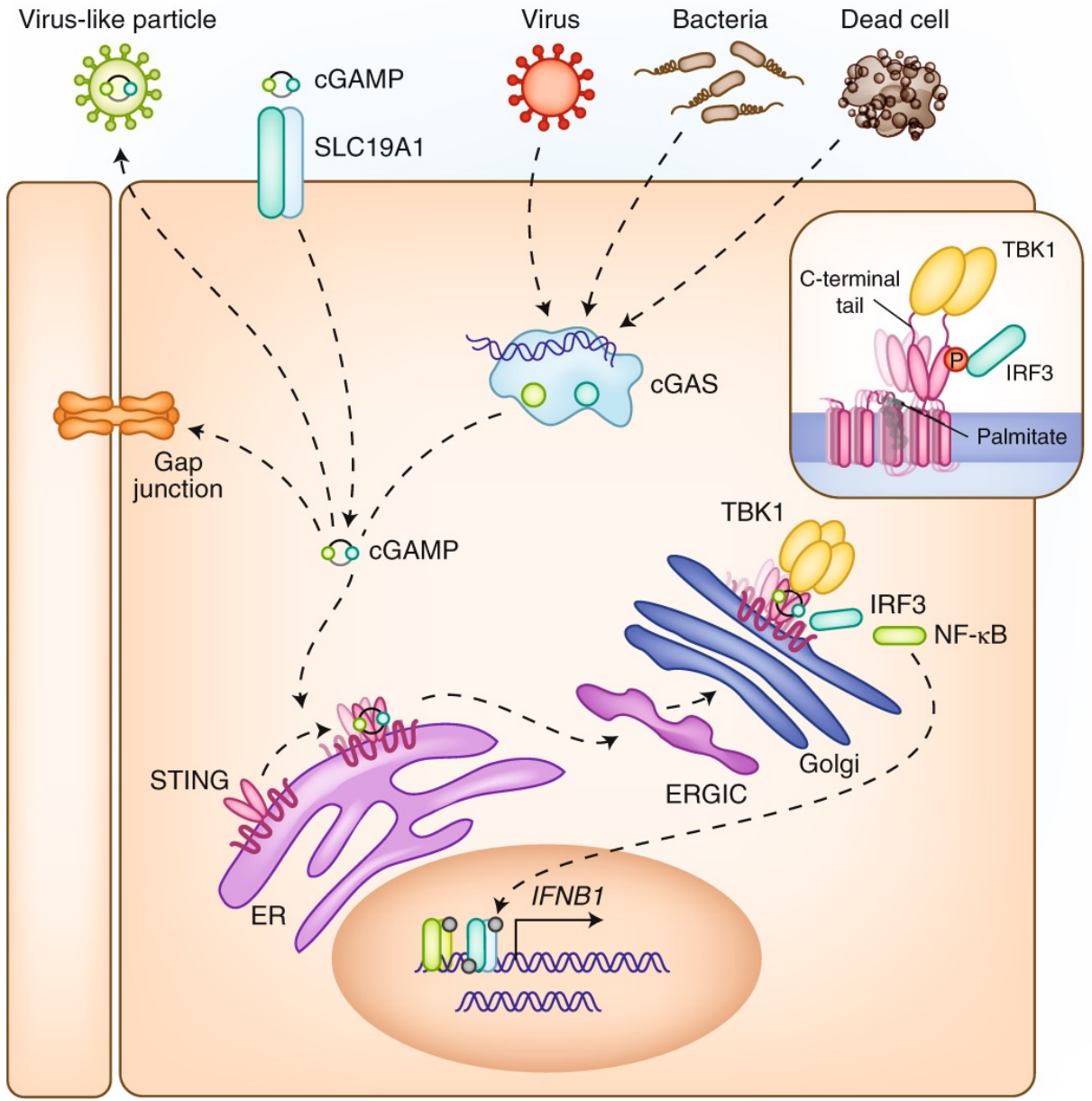


cGAS senses cytosolic DNA



RIG-I or **MDA5** can recognize RNA viruses

NLRP3 senses danger/stress (ATP, ROS, K+ efflux)


Virus-like particle
cGAMP
SLC19A1
Virus
Bacteria
Dead cell
TBK1
C-terminal tail
P
IRF3
Palmitate
cGAS
Gap junction
cGAMP
TBK1
IRF3
NF-κB
STING
ERGIC
Golgi
ER
IFNB1

BIO-479: Seminar 1

Activating the innate immune system from within

- An intact immune system activates upon presentation of external stimuli
- However, there are medical conditions characterized by excessive immune activation, or immune activation without an external stimulus: **Type 1 interferonopathies**

Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *The Year in Human and Medical Genetics: Inborn Errors of Immunity*

Type I interferonopathies: a novel set of inborn errors of immunity

Yanick J. Crow

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The concept of grouping Mendelian disorders associated with an upregulation of type I interferon is not currently recognized in the medical literature. Here, we argue that such a concept has scientific validity and clinical utility. Specifically, we discuss a group of conditions, including Aicardi–Goutières syndrome, spondyloenchondrodysplasia, and cases of systemic lupus erythematosus with complement deficiency, in which an upregulation of type I interferons is apparently central to their pathogenesis. We believe that these diseases can usefully be considered to represent a novel set of inborn errors of immunity, and that the recognition of such diseases as type I interferonopathies will have significance in the development and use of targeted therapies.

Keywords: type I interferon; interferonopathy; Aicardi–Goutières syndrome; spondyloenchondrodysplasia; systemic lupus erythematosus

Preferred citation: Crow, Y.J. 2011. Type I interferonopathies: a novel set of inborn errors of immunity. In "The Year in Human and Medical Genetics: Inborn Errors of Immunity I." Jean-Laurent Casanova, Mary Ellen Conley & Luigi Notarangelo, Eds. *Ann. N.Y. Acad. Sci.* **1238:** 91–98.

Review Article | Published: 20 October 2021

The type I interferonopathies: 10 years on

Yanick J. Crow & Daniel B. Stetson

Nature Reviews Immunology **22**, 471–483 (2022) | Cite this article

Aicardi-Goutieres Syndrome (AGS)

- 1988: Virologist Lebon & pediatricians Aicardi & Goutieres described elevated IFNα in the CSF of children affected by a genetic disorder that **resembled in utero-acquired viral infection**
- AGS was the first Mendelian disease to be associated with enhanced type I interferon signalling
- It appeared as if the children had acquired a viral infection, but **without the actual presence of an underlying infection**

IFN-alpha was detected in cerebrospinal fluid and/or sera from 7 of 8 patients […]. The secretion of IFN-alpha was **prolonged**, as shown by its presence at different times between birth and 5 years, and was not associated with IFN-gamma.

ELSEVIER

Journal of the Neurological Sciences
Volume 84, Issues 2–3, April 1988, Pages 201-208

Intrathecal synthesis of interferon-alpha in infants with progressive familial encephalopathy

Pierre Lebon [1], Jean Badoual [2], Gérard Ponsot [2], Françoise Goutières [4], Françoise Hémeury-Cukier [3], Jean Aicardi [4]

"Virological investigations **excluded various congenital infections**."

A Progressive Familial Encephalopathy in Infancy with Calcifications of the Basal Ganglia and Chronic Cerebrospinal Fluid Lymphocytosis

J. Aicardi, MD, and F. Goutières, MD

Eight infants developed a progressive disorder of the central nervous system with bilateral spasticity and dystonia, acquired microcephaly, and a rapid course toward profound deterioration and death. All the patients had abnormal cerebrospinal fluid with mild but persistent lymphocytosis. Computed tomography showed various combinations of bilateral symmetrical calcifications in the basal ganglia, progressive brain atrophy, and deep white matter hypodensities, the first two being present in all families but not in every individual patient. The disorder is familial and probably genetic in origin, although some features, especially the pleocytosis, may erroneously suggest an inflammatory condition.

Aicardi J, Goutières F: A progressive familial encephalopathy in infancy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis. Ann Neurol 15:49–54, 1984

What could cause an infection-like response without an infection?

Letter | Published: 16 July 2006

Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 cause Aicardi-Goutières syndrome at the *AGS1* locus

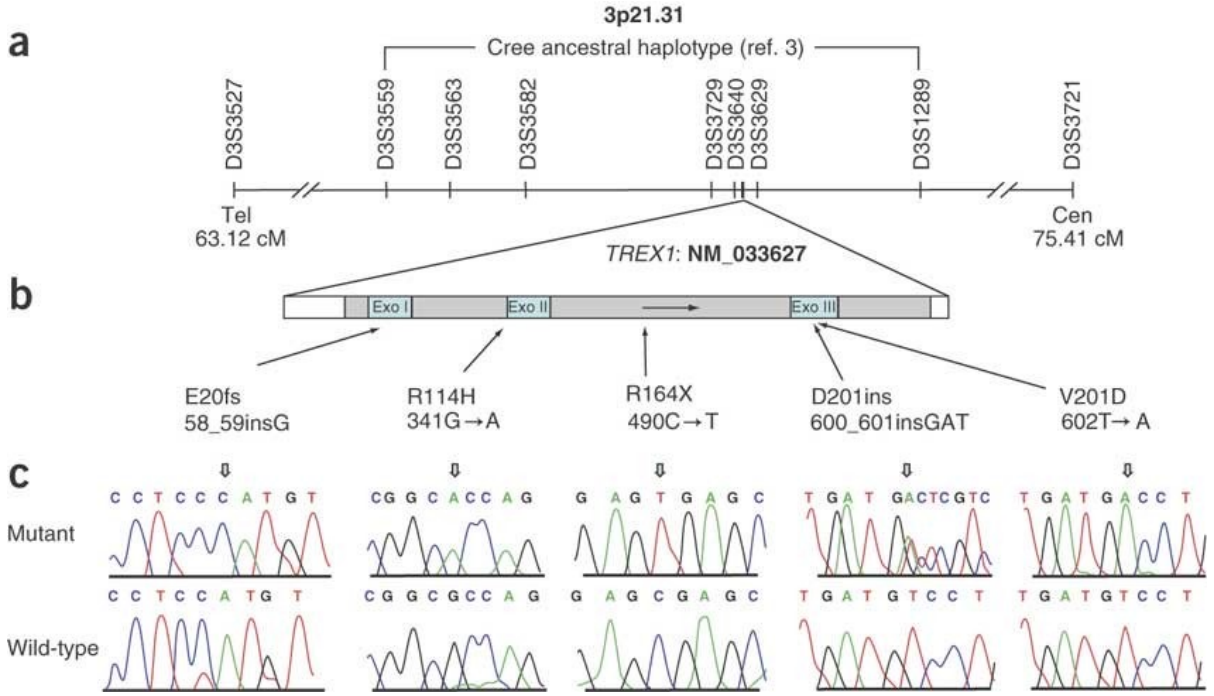
Yanick J Crow ✉, Bruce E Hayward, Rekha Parmar, Peter Robins, Andrea Leitch, Manir Ali, Deborah N Black, Hans van Bokhoven, Han G Brunner, Ben C Hamel, Peter C Corry, Frances M Cowan, Suzanne G Frints, Joerg Klepper, John H Livingston, Sally Ann Lynch, Roger F Massey, Jean François Meritet, Jacques L Michaud, Gerard Ponsot, Thomas Voit, Pierre Lebon, David T Bonthron, Andrew P Jackson, Deborah E Barnes & Tomas Lindahl — Show fewer authors

Nature Genetics **38**, 917–920 (2006) | Cite this article

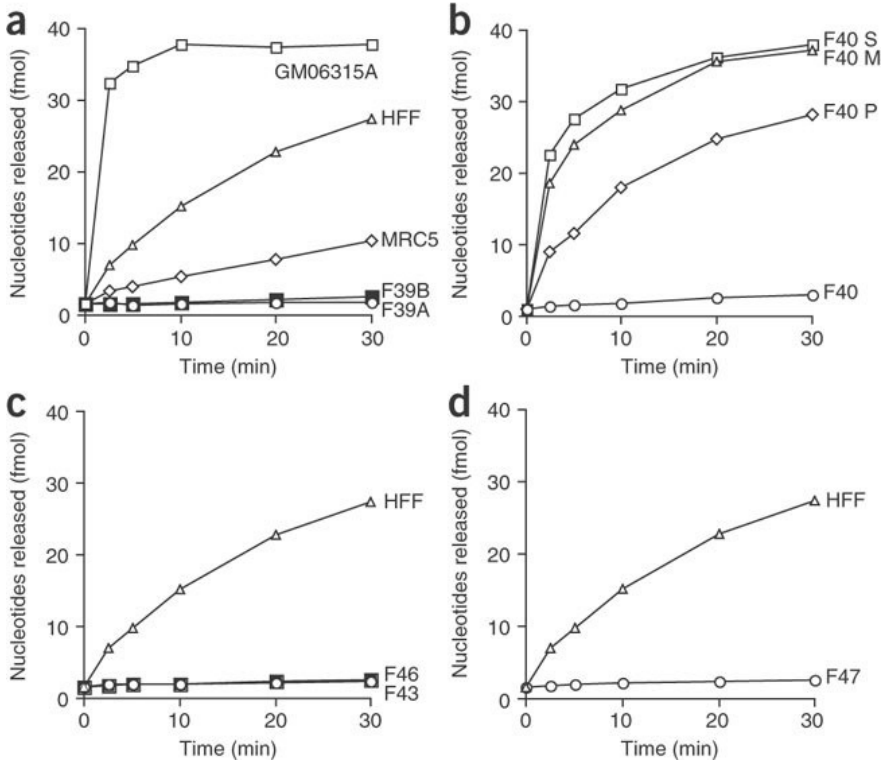
Genetic sequencing had delineated this locus as likely causal, however there was no clear link between the nearest gene (TREX1) and the immune phenotype of AGS

TREX1 is a 3′ → 5′ DNA exonuclease, which chews up free DNA in the cytosol (e.g. DNA of viral origins)

Mutations within TREX1 locus in AGS patients

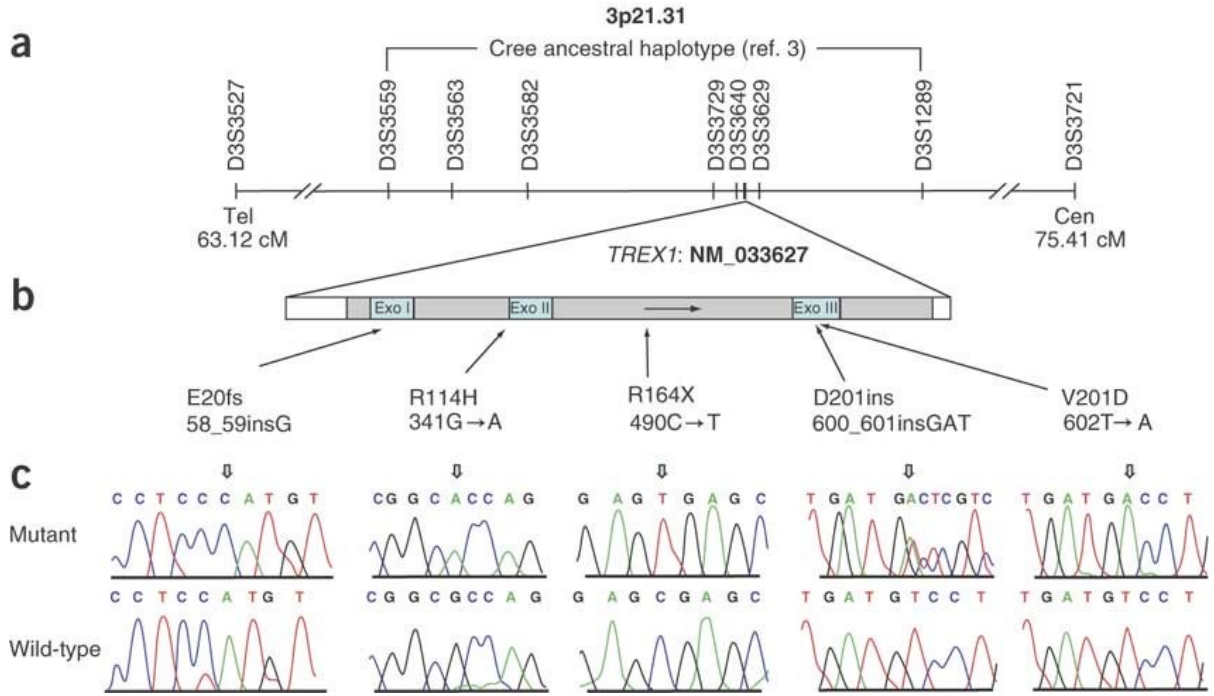


3' DNA exonuclease activity assay



- These two panels represent the entirety of the data in this paper (Nature Genetics 2006)
- Let's try to interpret them:

Mutations within TREX1 locus in AGS patients



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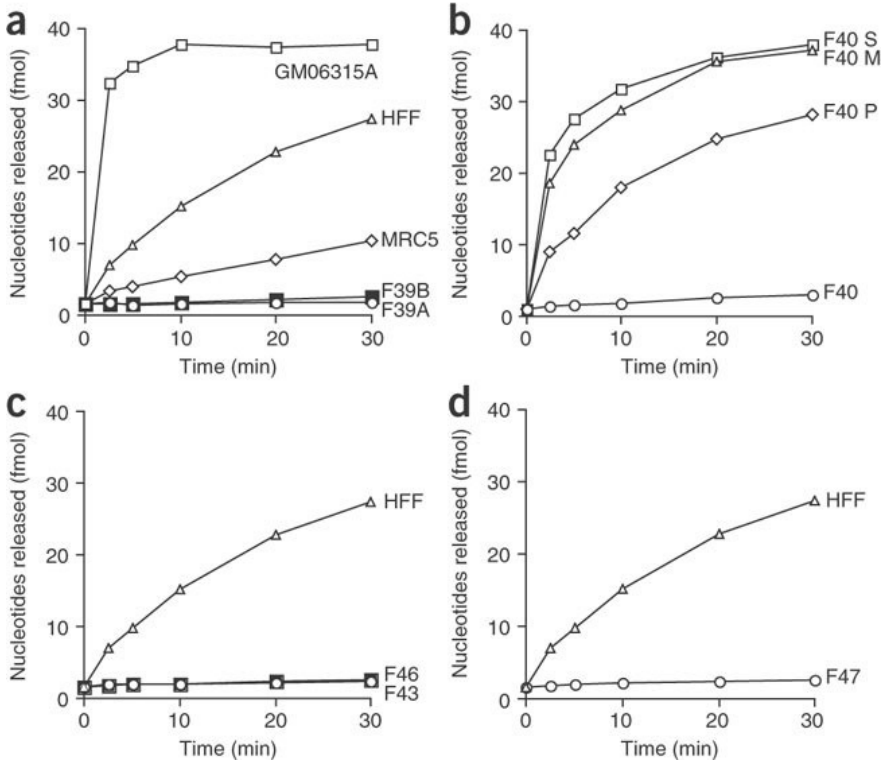
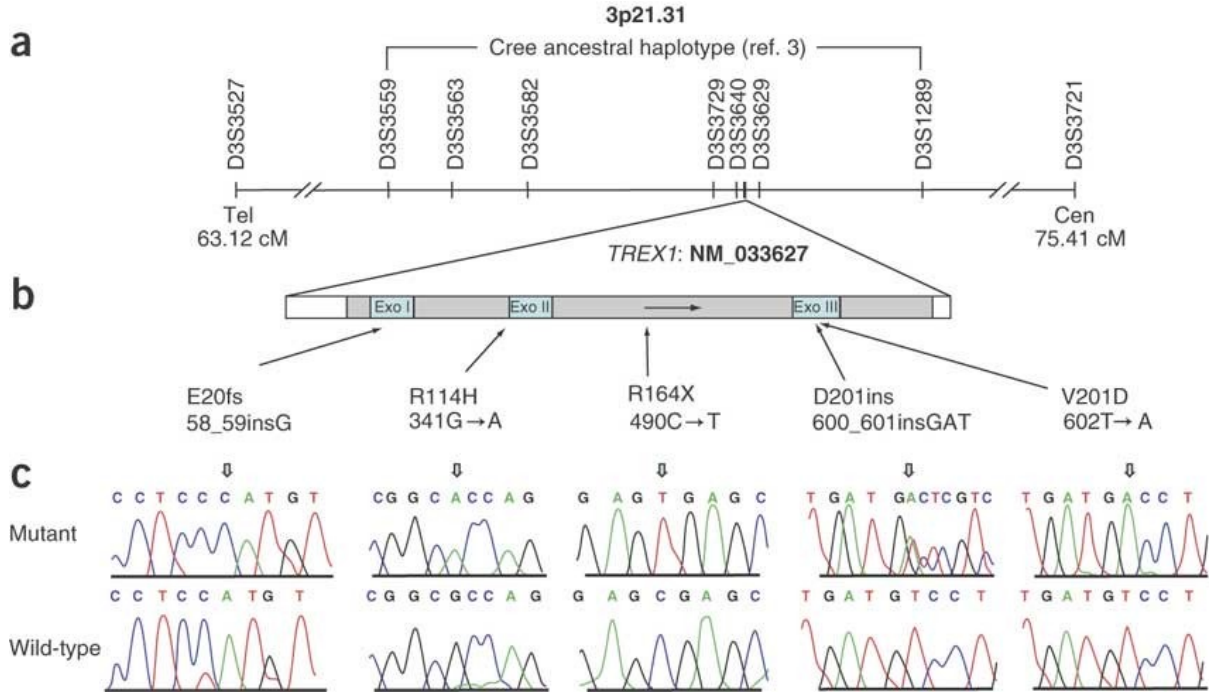


Cell-free protein extracts were assayed for 3′ DNA exonuclease activity with a 3′-end-labeled poly(dA) substrate

Sample Table
GM06315A: normal lymphoblastoid ctrl
MRC5: fibroblast cell line (no AGS)
HFF: fibroblast cell line (no AGS)
F39A: 341G → A
F39B: 341G → A + [600+601insGAT]
F40: 490C → T
F43: 341G → A
F46: 341G → A
F47: 602T → A

Mutations within TREX1 locus in AGS patients



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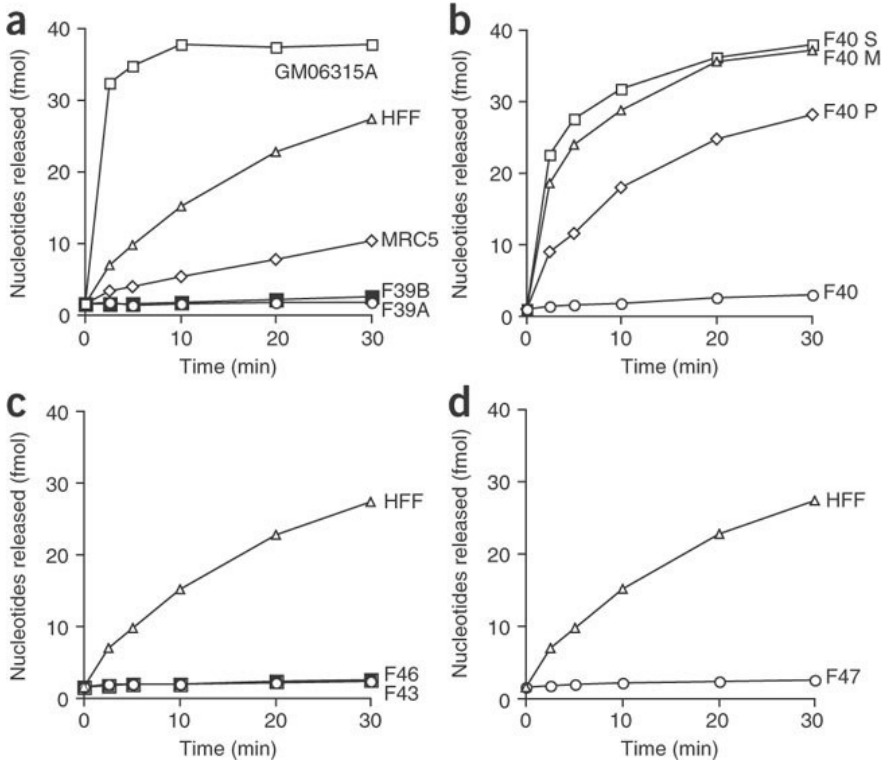

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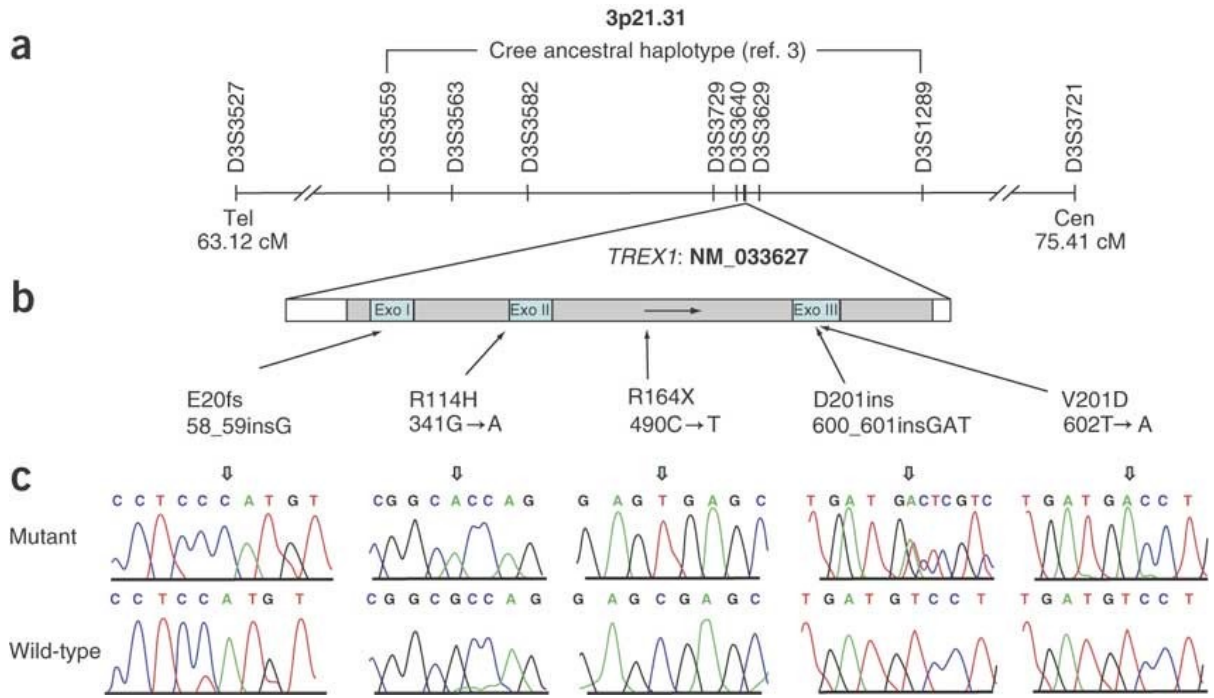
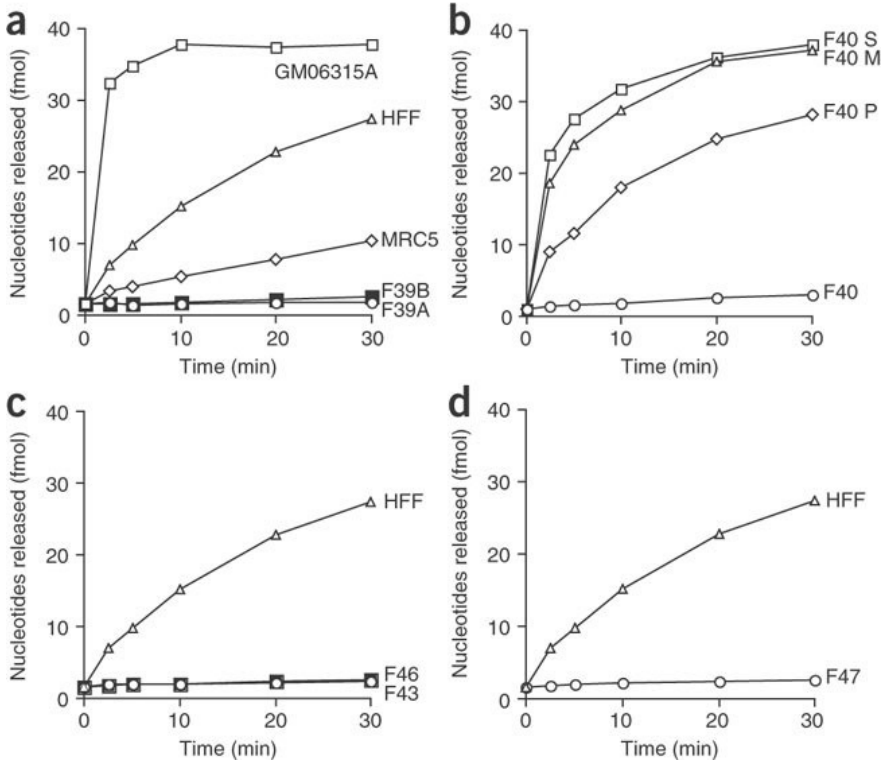
F40: 490C → T
F43: 341G → A
F46: 341G → A
F47: 602T → A

What conclusion would you draw from this data?

Mutations within TREX1 locus in AGS patients



3' DNA exonuclease activity assay



→ **The ability to degrade free DNA is impaired in AGS patients**
→ **These patients have mutations within a gene encoding for an exonuclease (TREX1), suggesting a causal link**

But why would a dysfunctional exonuclease lead to an immune response in the absence of infection?

- What would your hypothesis be?

Cell, 2008

Trex1 Prevents Cell-Intrinsic Initiation of Autoimmunity

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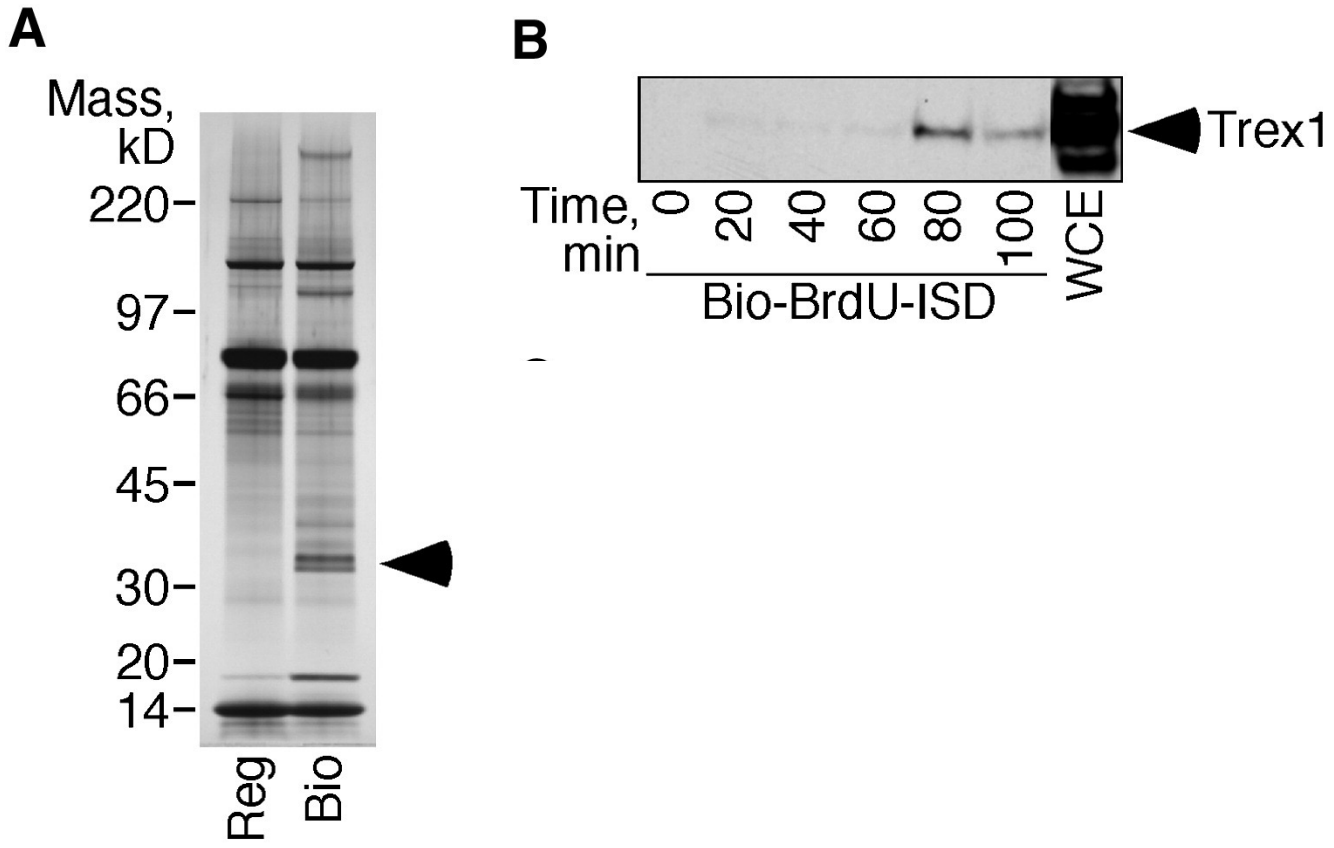
DOI 10.1016/j.cell.2008.06.032

Hypothesised that there are self-derived nucleic acids that can activate our innate immune receptors, and TREX1 usually keeps these in check

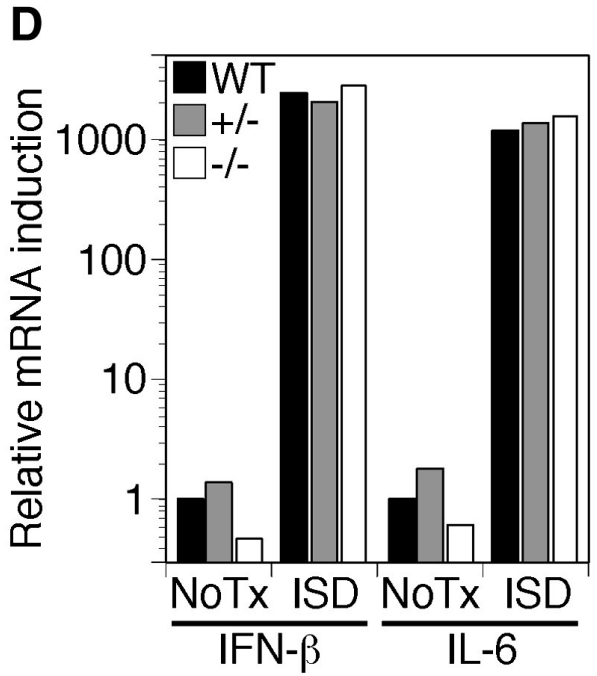
"[…] it is unclear whether accumulation of self-nucleic acids within cells can activate cytosolic sensors."

"[…] a number of fundamental questions regarding the principles of self-/non-self discrimination arise. Specifically, are there dedicated mechanisms that limit excessive activation of cytosolic nucleic acid sensors?"

Their first finding: TREX1 can bind externally delivered immuno-stimulatory DNA



Based on this data, is TREX1 the sensor of immunostimulatory DNA?



Bone marrow-derived macrophages of the indicated *Trex1* genotype were transfected with ISD. IFNβ and IL-6 mRNA expression 4 hr posttransfection were measured by quantitative RT-PCR, normalized to HPRT expression within each sample, and compared to untreated controls to calculate the relative expression.

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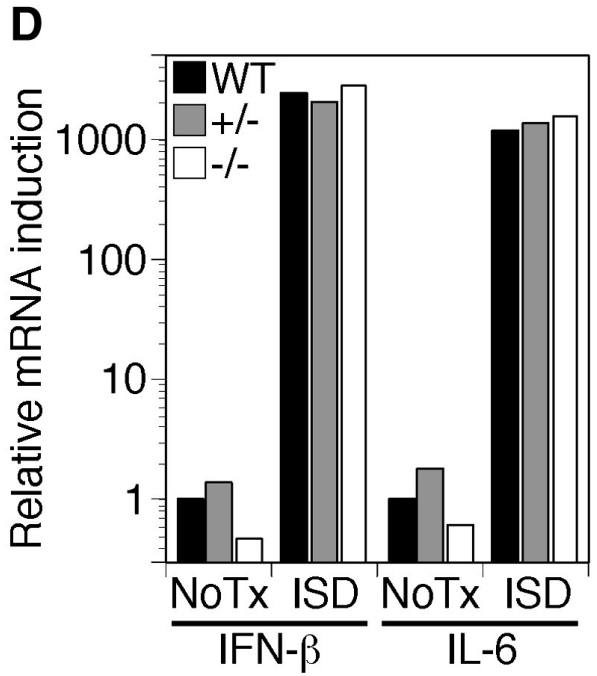

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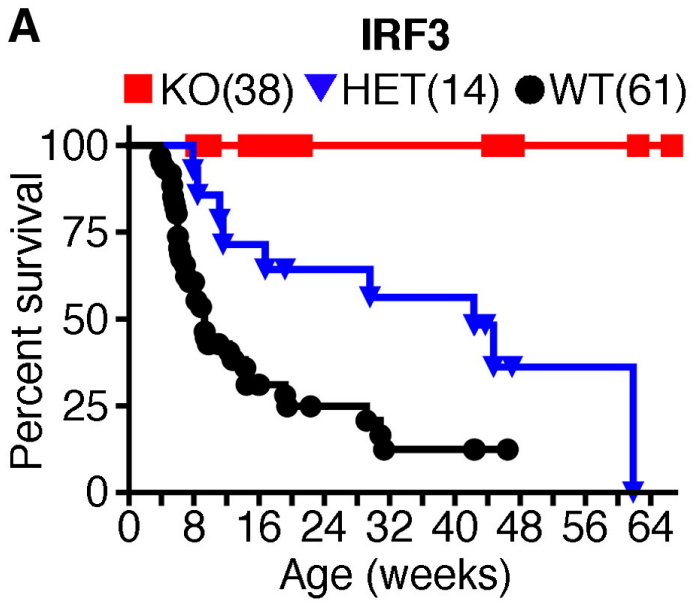
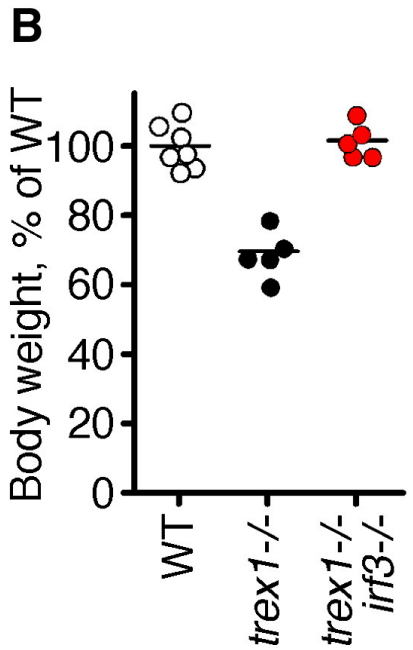
"We found that Trex1-deficient cells had an intact ISD response to transfected DNA, thus ruling out the possibility that Trex1 is the ISD sensor itself."

Genetic Dissection of the Trex1 Knockout Phenotype





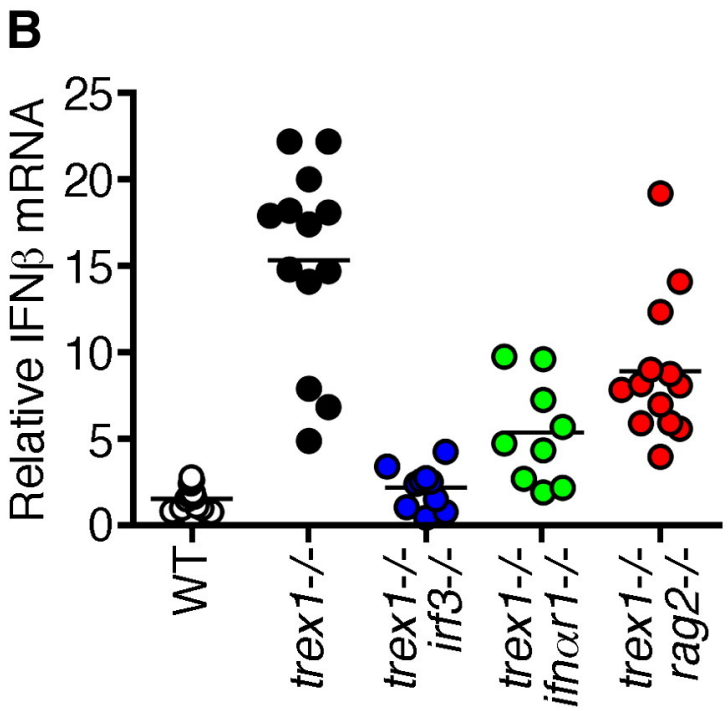
How would you describe the phenotype of knocking out TREX1?

How would you describe its relationship to IRF3?

What novelty does this finding offer regarding the role of TREX1?

(A) Survival curves for Trex1-deficient mice of the indicated *irf3* genotype. *Trex1*$^{-/-}$ *irf3*$^{+/+}$ mice include mice generated both by intercrossing *trex1*$^{+/-}$*irf3*$^{+/-}$ mice as well as by separately intercrossing *trex1*$^{+/-}$*irf3*$^{+/+}$ mice. The number of mice of each genotype is indicated in parentheses, and symbols represent individual mice.

(B) Body weights of mice of the indicated genotype were measured at 4.5 weeks of age and compared to the average weight of sex-matched, WT littermates. For these measurements, *trex1*$^{+/-}$ mice were considered WT.



(B) IFNβ mRNA levels in hearts of mice of the indicated genotype were determined by quantitative RT-PCR, normalized to HPRT expression within each sample, and compared to WT littermates to calculate the relative IFNβ expression.

What information does this data give us?

Does this demonstrate a novel finding, or does it back up one of the previously made points?

KO of TREX1 leads to immune sensor activation

- **“The data presented above suggest that accumulation of Trex1 DNA substrates activates the ISD pathway, raising questions about the nature and the source of this DNA.”**
- What is the endogenous substrate which, upon TREX1 knockout, starts activating immune sensors ?!

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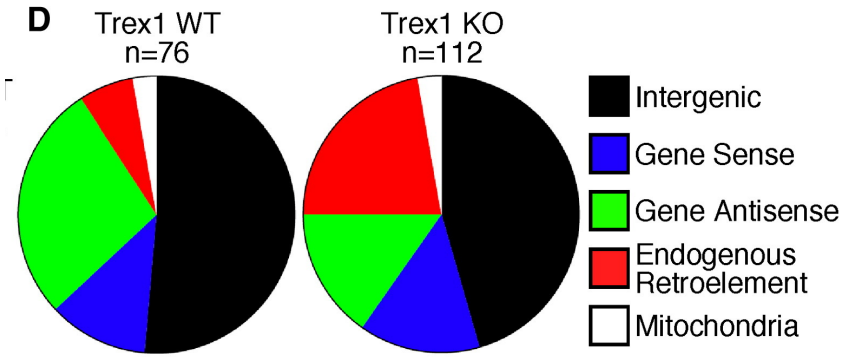

DNA was purified from pooled hearts of three *irf3*$^{-/-}$ mice and three *trex1*$^{-/-}$ *irf3*$^{-/-}$ mice. This plot represents the relative percentage of each type of DNA fragment.

Based on this data, can we answer the question?

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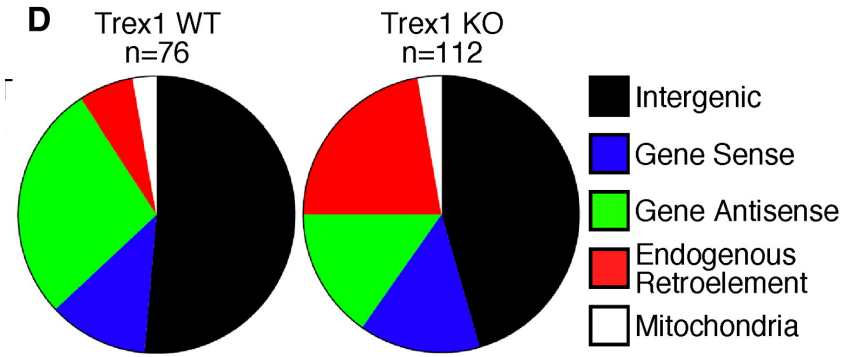
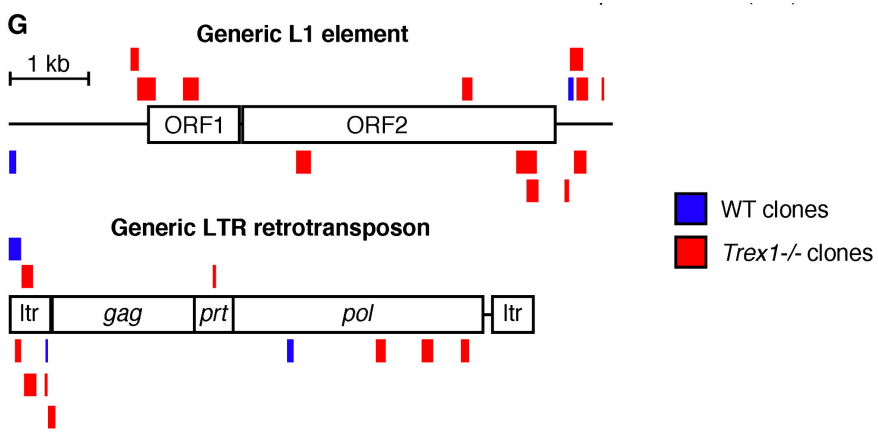


F

	WT	KO
Total clones	129	137
Poly-dT	53 (41%)	25 (18%)
Unique	76 (59%)	112 (82%)
Mitochondria	2 (3%)	3 (3%)
Genes Sense	9 (12%)	16 (14%)
Genes Antisense	21 (28%)	17 (15%)
Intergenic	39 (51%)	51 (46%)
Retroelements	5 (7%)	25 (22%)
LINE	2	12
SINE	0	3
LTR	3	10



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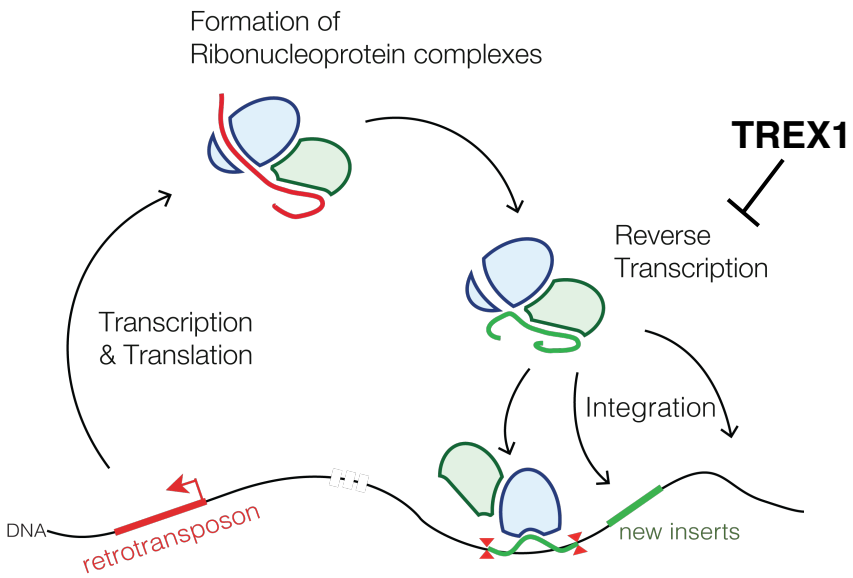
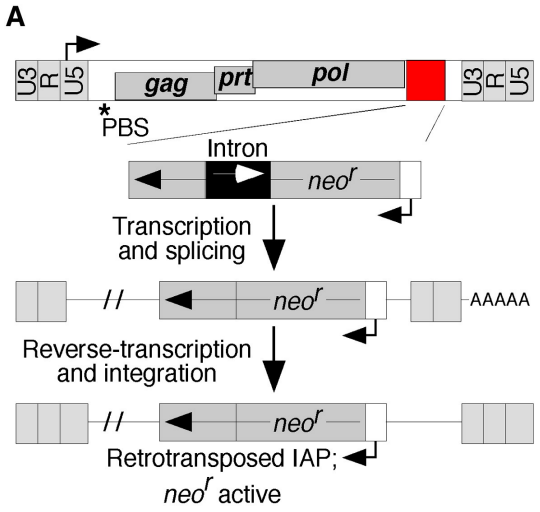
ssDNA fragments that mapped to endogenous retroelements were plotted to scale against a generic, consensus sequence of L1 (top) and LTR (bottom) elements. Sense fragments are above each element, and antisense matches are below. For the fragments that mapped to LTRs, only the 5′ mapping is shown to avoid redundancy.

An unexpected source of endogenous immunostimulatory nucleic acids: The reverse-transcribed cDNA of endogenous retroelements



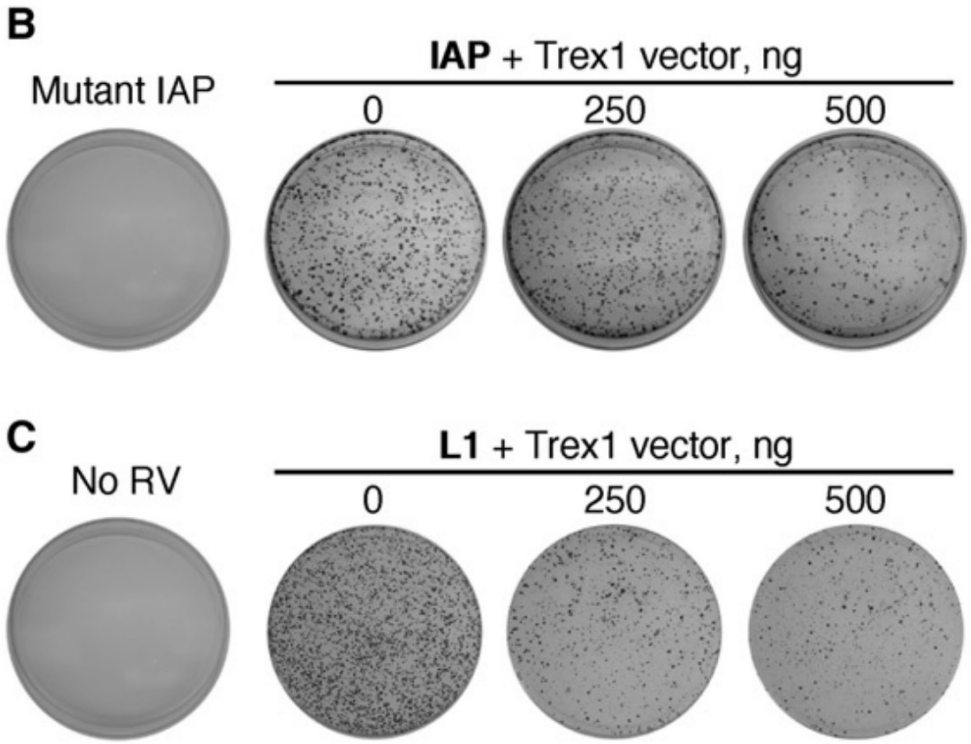
A final piece of data:

Reverse transcription reporter



- IAP = a type of endogenous retrovirus
- Each element carriers a neomycin-resistance gene in reverse orientation, and interrupted by an intron
- In this conformation, neo_r is non-functional
- Only if the element transcribes → splices out the intron → reverse transcribes → integrates: a functional neo_r copy appears

What does this piece of data prove?



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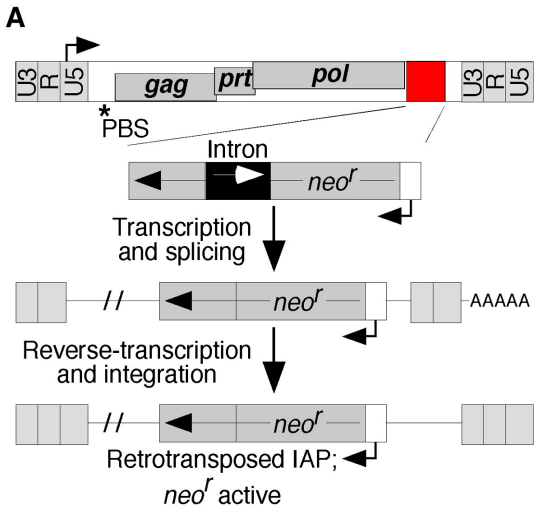

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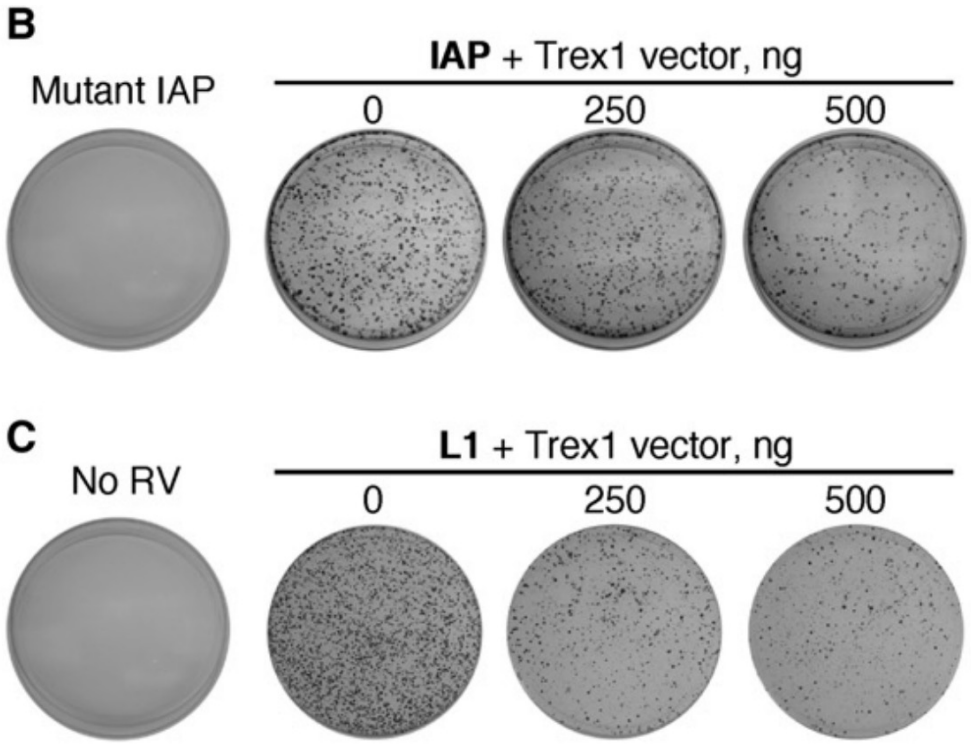

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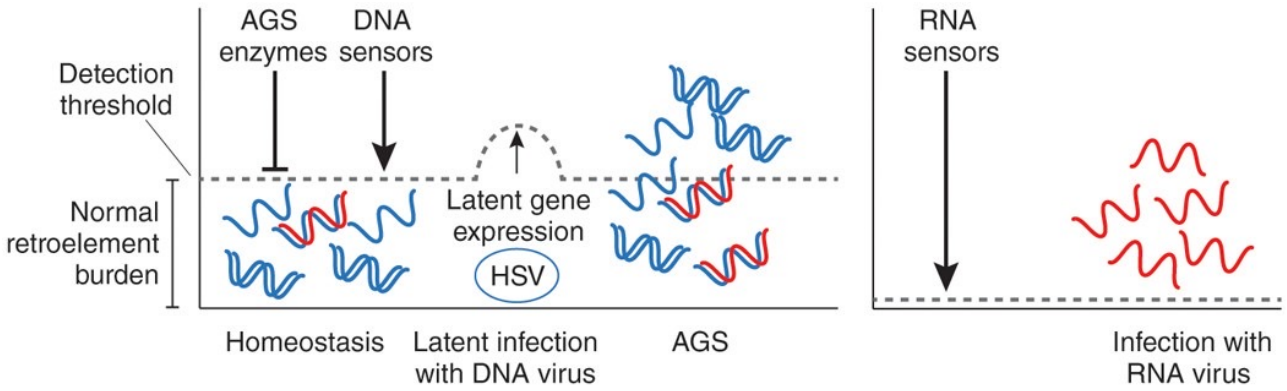

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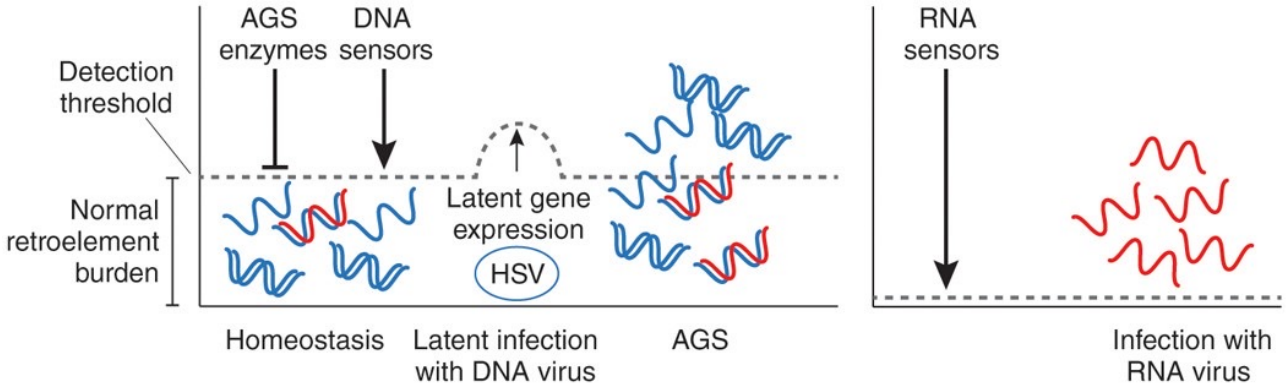
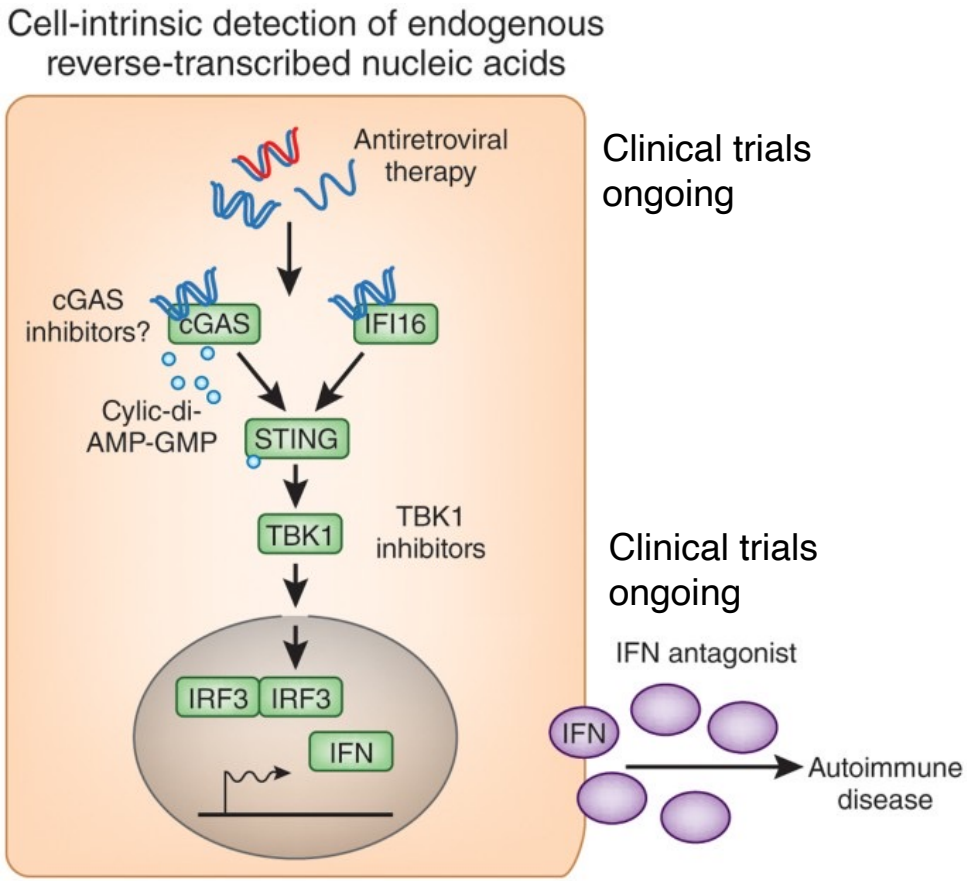


Clinical trials for other type I interferono-pathies



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