THE ONASSIS FOUNDATION SCIENCE LECTURE SERIES

The 2009 Lectures in Biology: IMMUNOBIOLOGY

Autoimmunity and Inflammation

From bench to bed-side

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Immunity and its homeostatic regulation

- The immune system is among the most fundamental requirements for survival. Thus, it not surprising that many pathogen-sensing systems and immune pathways are *evolutionary conserved throughout the species*.

- A basic problem confronting all living organisms including the humans is how to defend against foreign invasion or factors that may disturb its basal state (homeostasis) while *maintaining control of the defending forces (homeostatic regulation).*

- Many of the *human diseases* are now thought to be the result of *dysfunctional innate and/or adaptive immune responses to external pathogens or endogenous molecules derived from a "stressed host*".

- These are collectively called stress associated molecular patterns (SAMPs) and include among others products of apoptotic or necrotic cells, metabolic products, and more recently even nutrients.

Inflammation and autoimmunity

- ***Inflammation*-an adaptive response triggered by a variety noxious stimuli and conditions, triggers the recruitment of leukocytes and plasma proteins to the affected tissue site;**

  -Inflammation underlies many of the human diseases associated with the immune system. The list keeps expanding to include common diseases initially not thought to be inflammatory but degenerative

- ***Autoimmunity*-the recognition of self-constituents by the immune system, can result *in tissue dysfunction and pathology with or without inflammation***

- **In contrast to infectious diseases, in inflammatory and/or autoimmune diseases, the production of inflammatory cytokines and the resultant systemic inflammation are thought to be induced by endogenous molecules (SAMPs)**

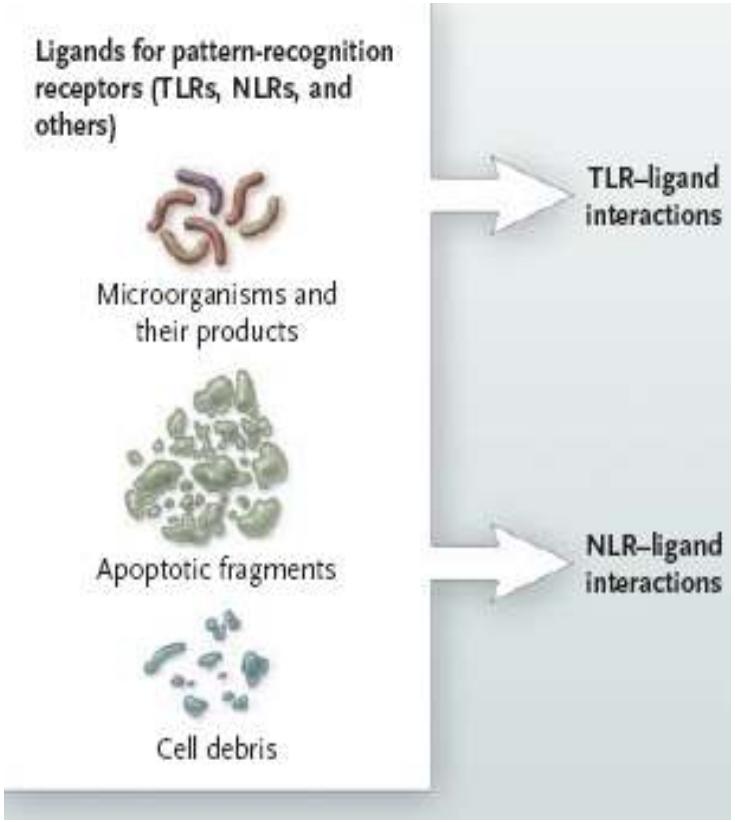
- **The realization that there is a *cross-talk between the innate and the specific immune response* has motivated investigators in recent years to take *a closer look at the contribution of innate immunity in these diseases*.**

The immune system has sensors not only for the pathogens but also for other stressors : PAMPs+SAMPs =Damage AMPs (DAMPs)

- **These sensors (TLRs, NLRs) recognize**

  -pathogens (pathogen associated molecular patterns, PAMPs) ή

  -host-derived stress (stress associated molecular patterns, SAMPs).



Host-derived stress signals: by-products of apoptotic cells (hsp, histonic proteins, HMGB1, DNA, RNA, uric acid), metabolic products or nutrients (free fatty acids, cholesterol, ATP, glucose etc)

Immunity, Inflammation and Autoimmunity in Humans

Physiology, pathophysiology, nosology and therapeutics

- **Origin and physiologic role of inflammation**
- ***Exogenous*** **inflammation vs** ***endogenous*** **inflammation and associated diseases**
- ***Endogenous inflammation*****: Auto-inflammation vs autoimmune inflammation**
  - **Auto-inflammatory diseases: Diseases of innate immunity**
  - **Autoimmune diseases: Diseases of innate and adaptive immunity**
- **Biologic therapies: Lessons learned about the targeting of key molecules and cells**
- **Inflammatory and autoimmune diseases are complex: the use of high-throughput methods in their investigation**
- **Perspective**

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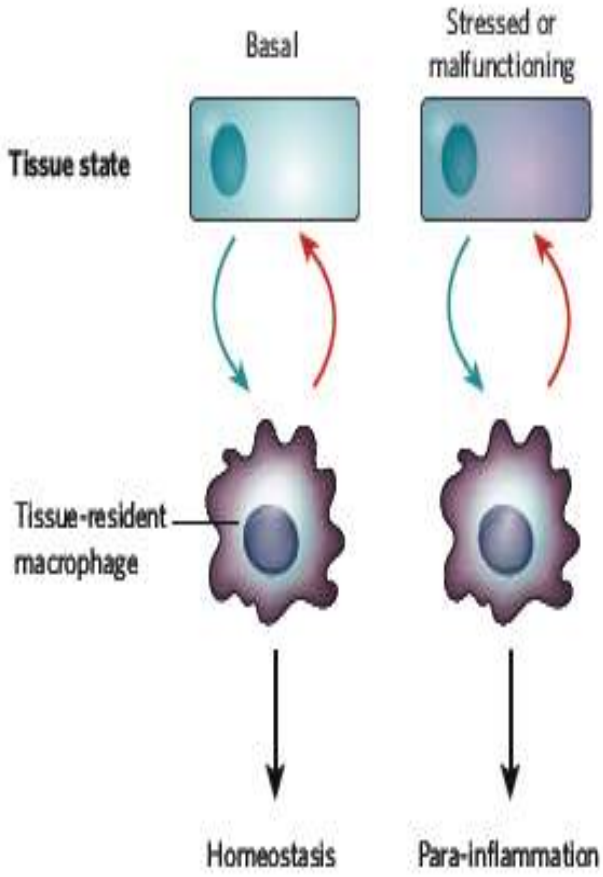
Inflammation underlies a wide variety of physiological and pathological processes.

Although the pathological a spects of many types of inflammation are well appreciated, their physiological functions are mostly unknown

Cellular states and surveillance of tissues by MΦ

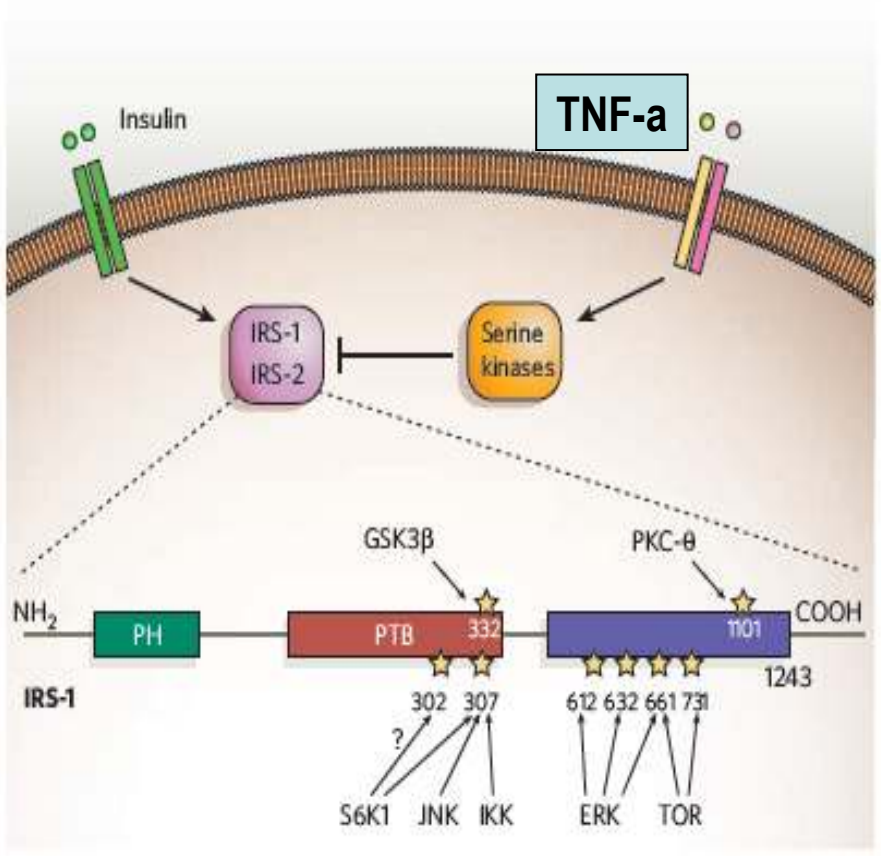


Blue arrows indicate signals that report the tissue state to MΦ;
Red arrows indicate MΦ -derived signals that control tissue adaptation

Surveillance of the tissues is important for homeostasis
Para-inflammation in the adipose tissue (obesity) and its relation to T2DM

- **Mediated by tissue macrophages and mast-cells**
- **They control/monitor/mediate the removal of apoptotic cells, the integrity and proliferation of the epithelial surfaces, the production of mediators from the adipocytes and the remodeling of the tissues.**
- **When the stress of the cells exceeds their limits, adipo-cytes secrete CXCL12 which attracts new macrophages**
- **The increased number of macrophages and the resultant increase in inflammatory cytokines such as Il-1 and TNF-a increase the resistance to insulin which promotes atherosclerosis**
- **Insulin resistance affects predominantly muscles and the fat so that the glucose is available for WBCs to fight the infection**

Insulin-receptor signalling interfaces with inflammatory signalling at the level of insulin-receptor substrates through activation of serine kinases.



TNF blocks insulin signaling

Basal state (Homeostasis)

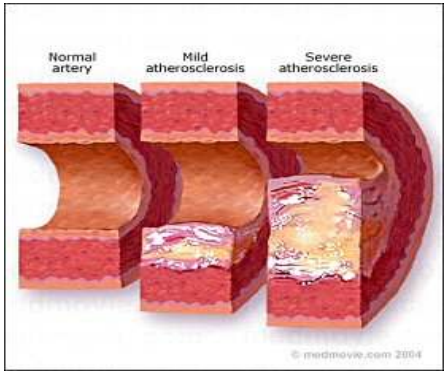
Stress
-lipids
-hypertension
-oxidation
-hypoxia
-ageing

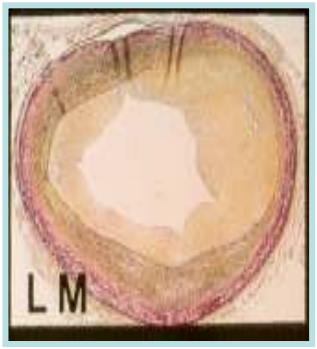
Tissue surveillance by macrophages

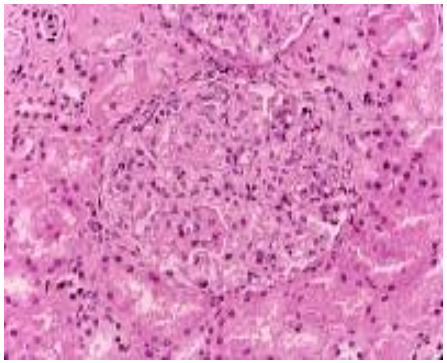
Infection
Tissue injury
Auto-immunity
Auto-inflammation

Para-inflammation
Increase in MΦ- plasma proteins
Endothelial dysfunction

Inflammation
Infiltration by inflammatory cells
Monocytes, lymphocytes



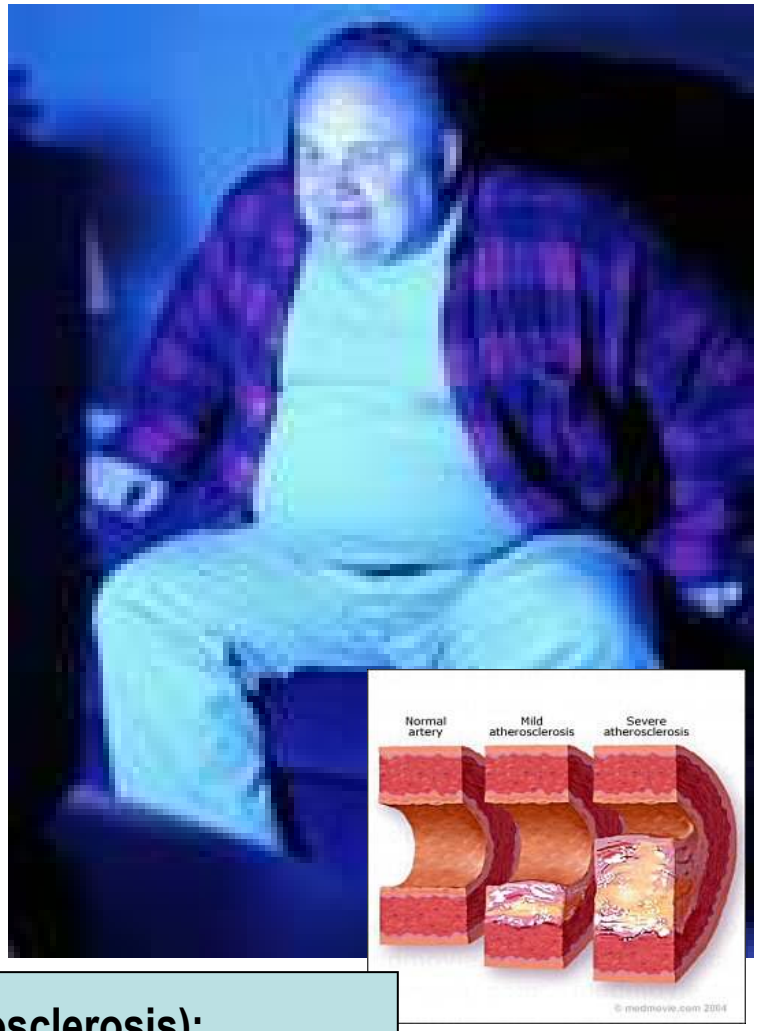




Low-grade inflammation in metabolic obesity, T2DM and atherosclerosis

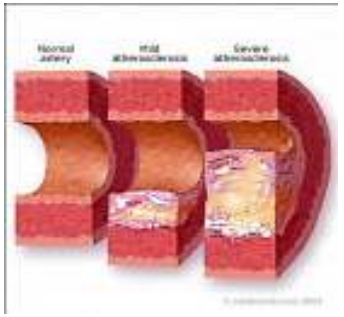
Atherosclerosis and T2DM represent inflammatory diseases

- **The body has not been adapted yet to states such as obesity, lack of exercise, smoking, hyperglycemia, atherosclerosis, hypertension, ageing etc**
- **These low-grade inflammatory states are thought to represent a mal-adaptation to these conditions**
- **Increase number of macrophages and inflammatory cytokines such as IL-1 and TNF-a**
- **Proof of concept: Phase 2 clinical trials with inhibitors of IL-1(Anakinra) improves glycemic control in T2DM**



Metabolic syndrome (Risk factor for atherosclerosis): obesity, insulin resistance, dyslipidemia

Chronic inflammation promotes atherosclerosis



- **A common feature of all chronic inflammatory diseases is premature, accelerated atherosclerosis which represents a major cause of morbidity and mortality of these patients**

- **In rheumatoid arthritis patients with high-disease activity have resistance to insulin mediated by TNA-a which antagonizes the action of insulin at insulin.**

- **Ant-TNF treatment ameliorates insulin resistance by decreasing the phosphorylation at serine residues (Sidiropoulos et al)**

- **Aggressive control of atherosclerotic risk factors in these patients and aggressive control of inflammation**

The inflammatory pathway involves several components

***Inducers, sensors**, mediators, effectors*

Mediators: TNF, IL-1, IL-6,

Effectors: T cells, B cells, macrophages, neutrophils, other cells (epithelial cells, endothelial, mesenchymal cells, adipocytes

Key points

- **Inflammation underlies a wide variety of physiological and pathological processes.**
- **The classic instigators of inflammation — infection and tissue injury — are at one end of a large range of adverse conditions that induce inflammation, and they trigger the recruitment of leukocytes and plasma proteins to the affected tissue site.**
- **Tissue stress or malfunction similarly induces an adaptive response, which is referred to here as low-grade or para-inflammation.**
- **This response relies mainly on tissue-resident macrophages and is intermediate between the basal homeostatic state and a classic inflammatory response.**
- **Para-inflammation is probably responsible for the chronic inflammatory conditions that are associated with modern human diseases such as DM, atherosclerosis, degenerative diseases**
- **Inflammatory diseases are systemic diseases. TNF, IL-1 key cytokines**
- **Inflammation can be linked to cancer, fibrosis, degeneration, allergy and autoimmunity**

Inflammation: outstanding needs

- Need a more relevant definition of inflammation at the molecular and cellular level which

  -distinguishes inflammation from tissue injury/damage and repair/remodeling

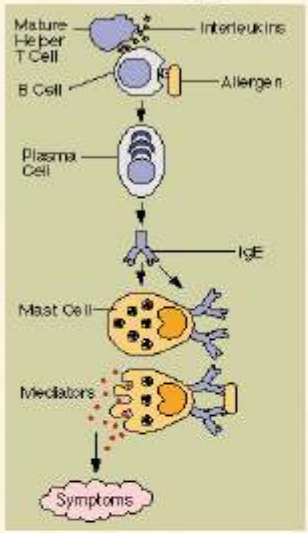
  - considers the full range of severity and frequency

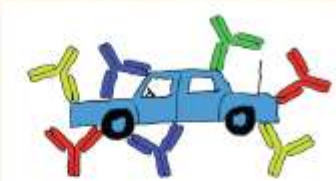
  - is more specific and more sensitive

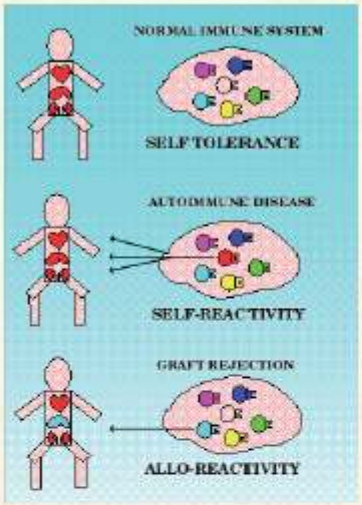
- Need for relevant biomarkers; the old biomarkers CRP, SAA not sensitive or specific

Dysfunction of the immune system has been linked to a variety of diseases

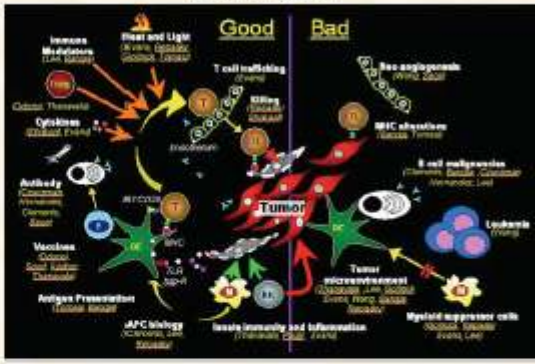
Allergy



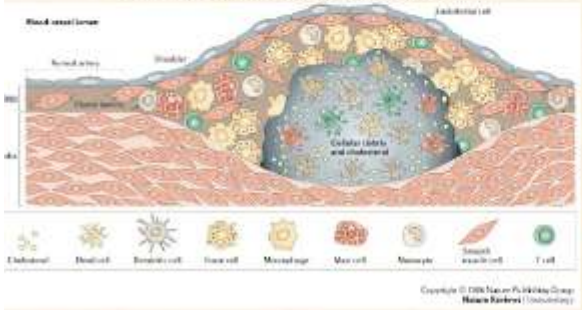




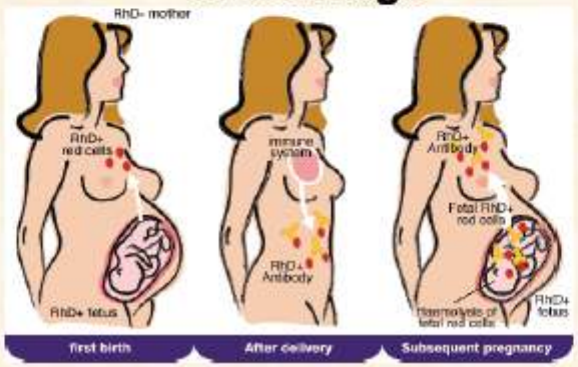
Cancer

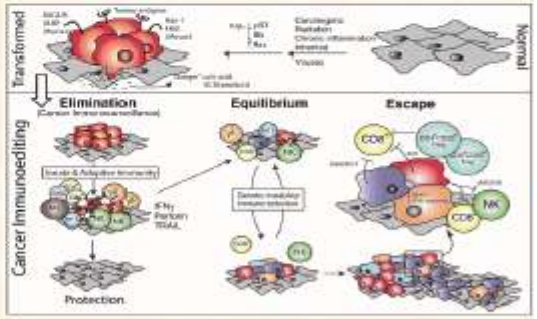


Atherosclerosis



Miscarriage





Immunity, Inflammation and Autoimmunity in Humans

Nosology

- ***Exogenous*** **inflammation vs** ***endogenous*** **inflammation and associated diseases**

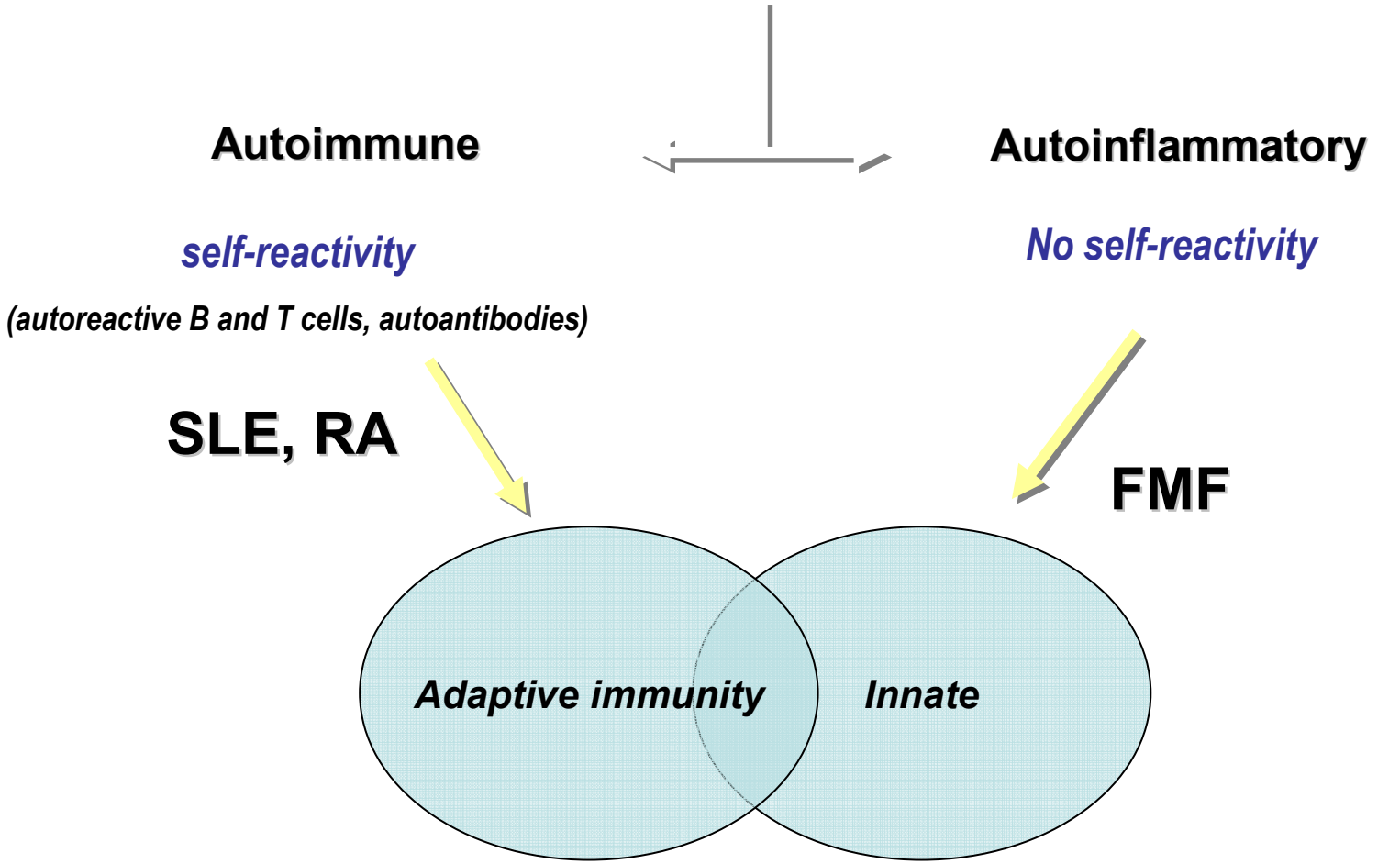
-Exogenous inflammation-associated diseases:

- infections
- allergens
- toxic exposure: drugs, chemicals, pollution, smoking,
- nutrients: gluten, ***cholesterol, glucose***

-*Endogenous inflammation*: Auto-inflammation vs autoimmune inflammation

- **Auto-inflammatory diseases: Diseases of innate immunity**
- **Autoimmune diseases: Diseases of innate and adaptive immunity**

Non-infectious inflammatory diseases from endogenous stimuli



Initiation – amplification – progression of the inflammatory response

Horror Autoinflammaticus

The Molecular Pathophysiology of Autoinflammatory Disease

The Molecular Pathophysiology of Autoinflammatory Disease.

- Initially coined by Kastner to describe FMF and TRAPS
- At present six categories of autoinflammatory disease

-IL-1β activation disorders (inflammasomopathies)

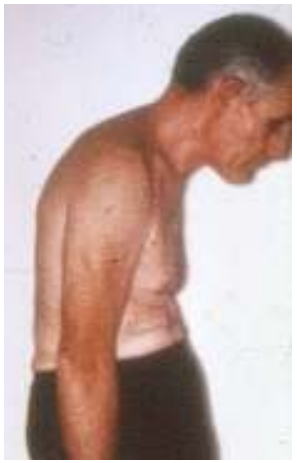
- NF-κB activation syndromes

-Protein misfolding disorders: AS, TRAPS

-Complement regulatory diseases,

-Disturbances in cytokine signaling

-Macrophage activation syndromes.



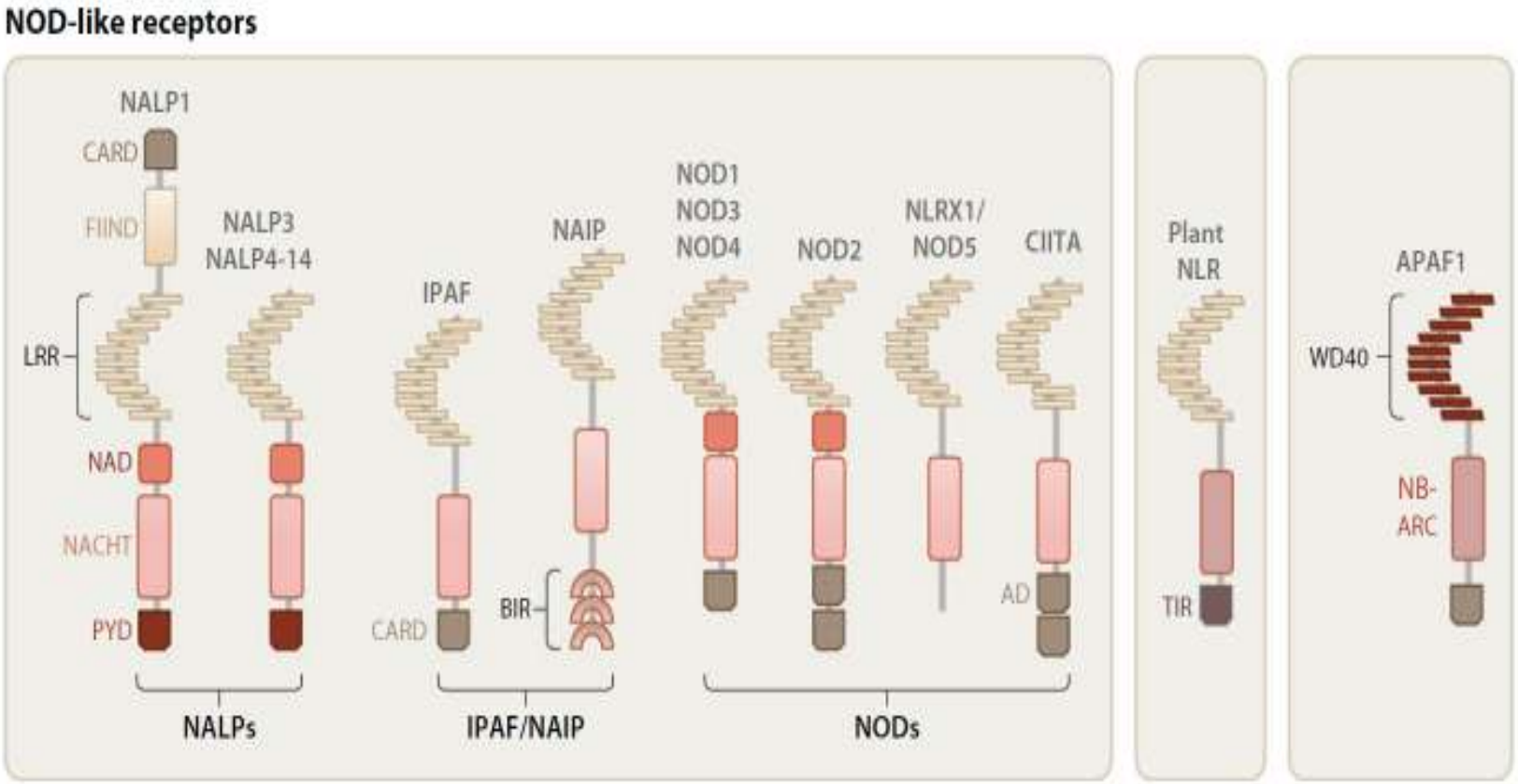
HLB27 MISFOLFING IN AS

Kastner DL et al Annu. Rev. Immunol. 2009. 27:621–68

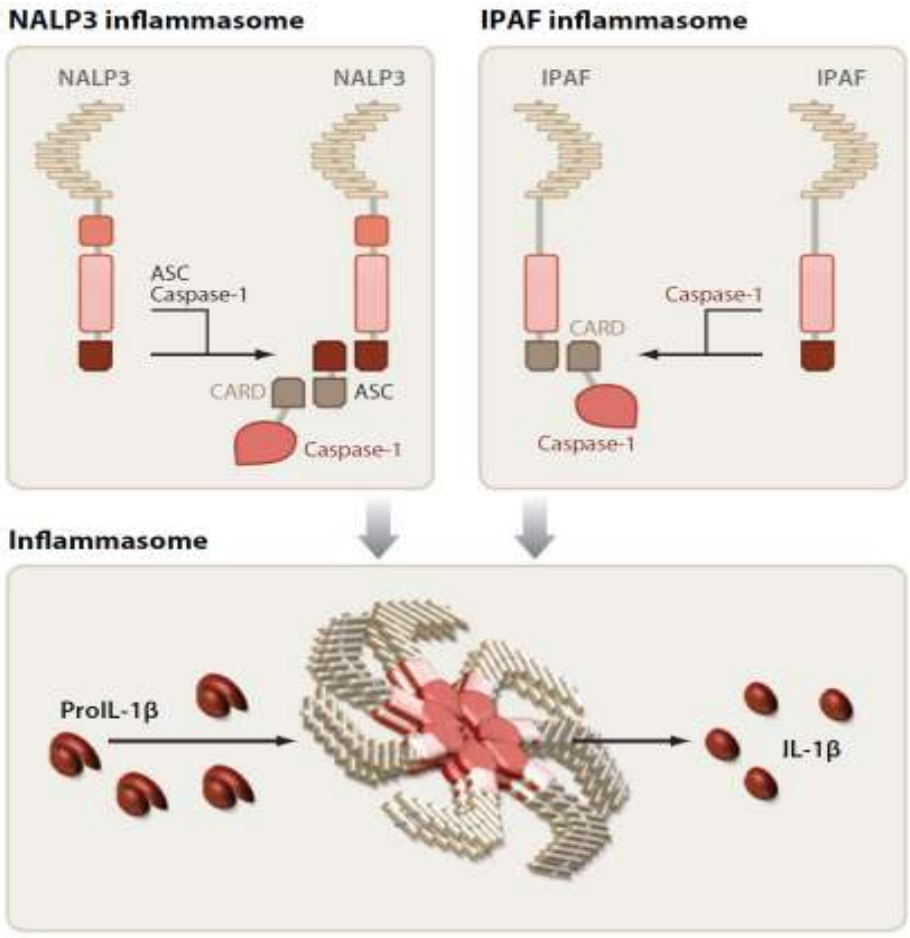
The Inflammasomes

Guardians of the body and inflammation engines

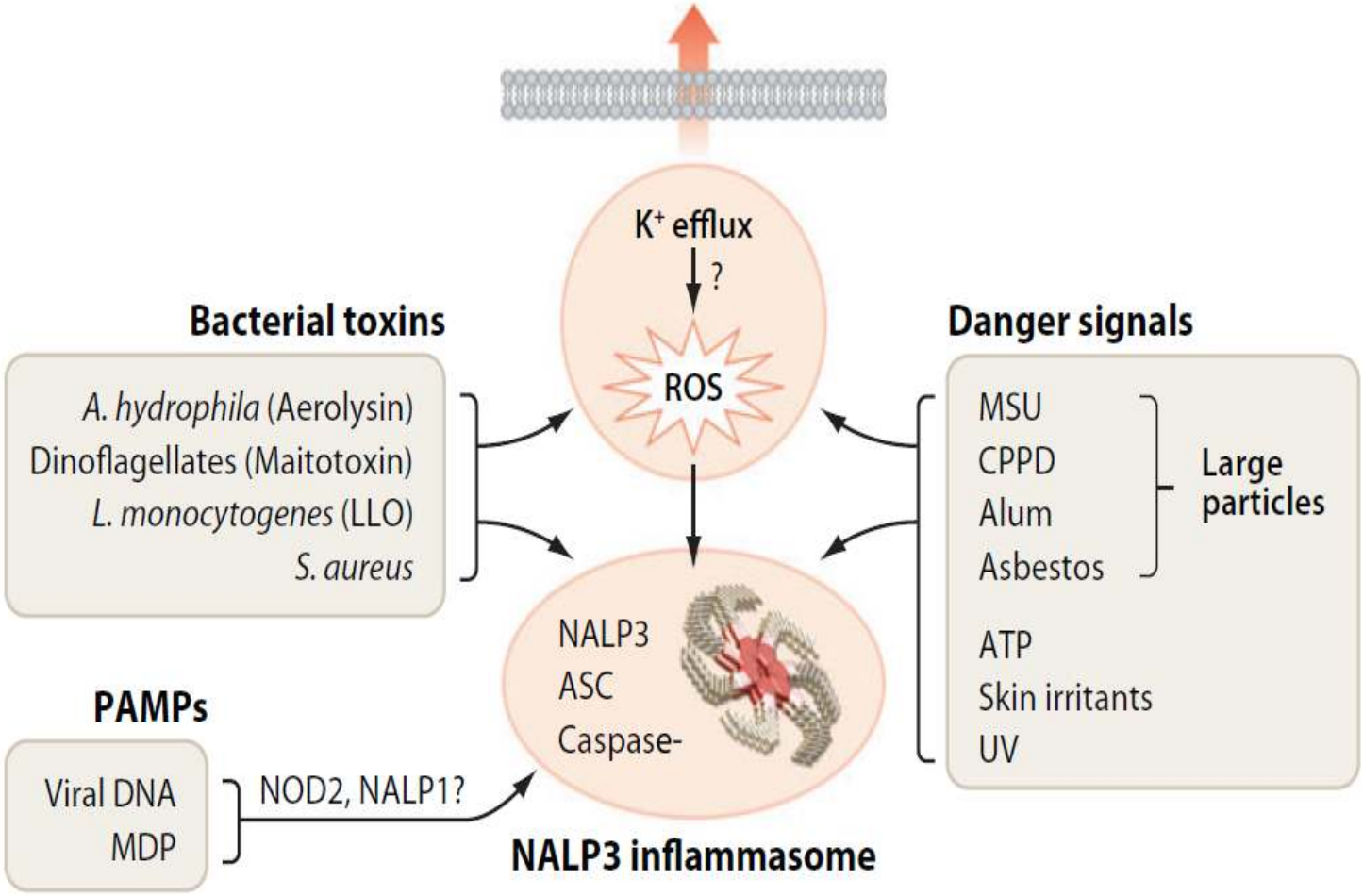
NOD-like receptors (NLRs) represent intracellular microbial sensors and physical and metabolic stress sensors



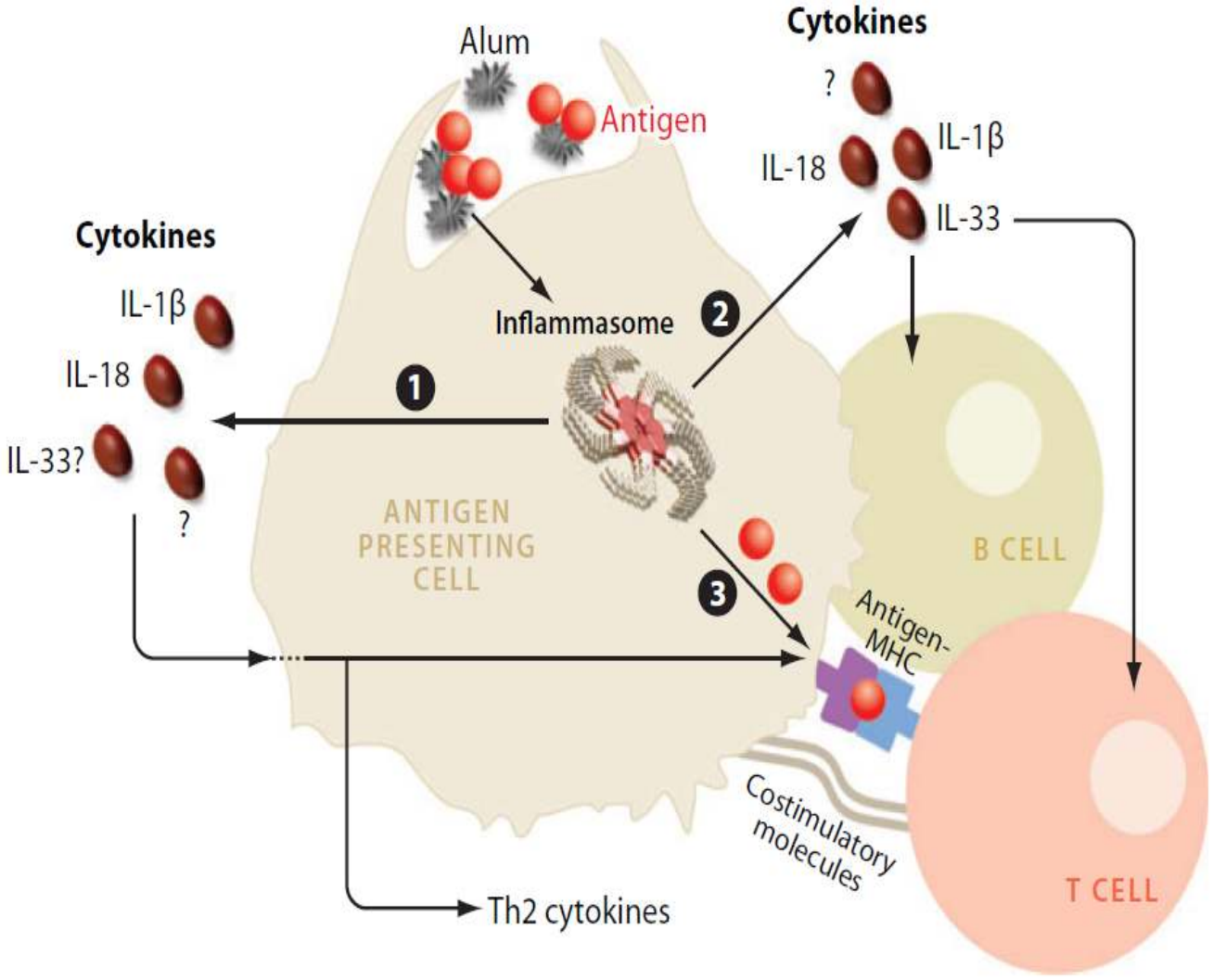
NLRs form large cytoplasmic complexes called inflammasomes that link the sensing to the proteolytic activation of the proinflammatory cytokines IL-1β and IL-18.



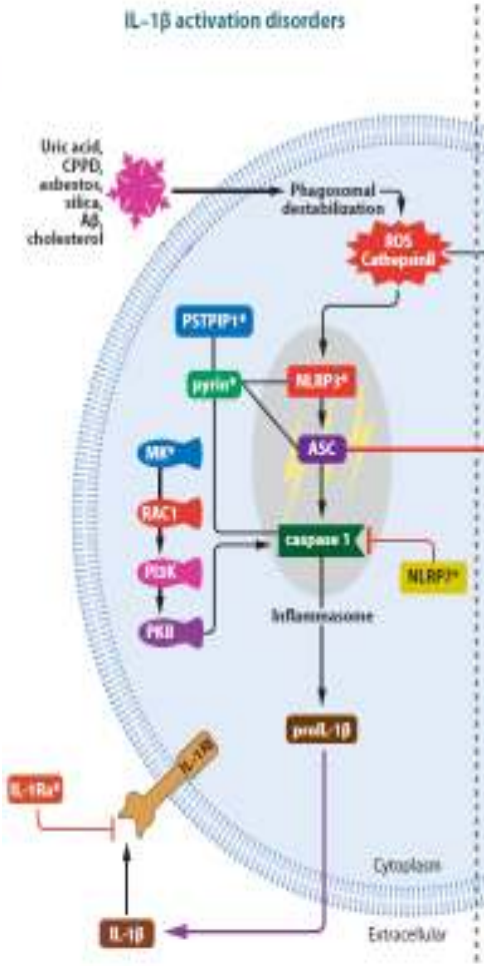
The NALP3 inflammasome has been associated with several autoinflammatory conditions including gout.

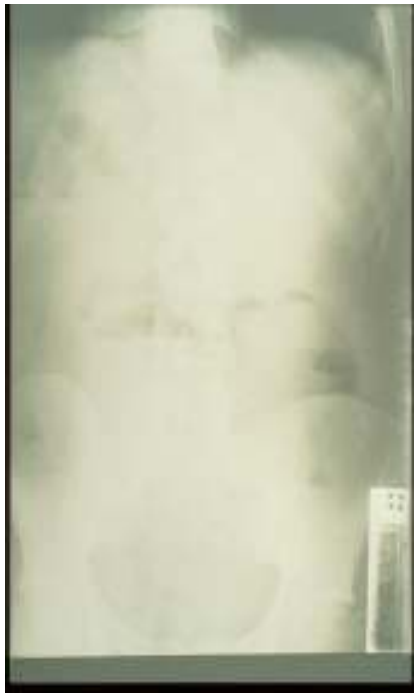


The NALP3inflammasome is a crucial element in the adjuvant effect of aluminum and can direct a humoral adaptive immune response



Disease	Gene (chromosome)	Protein (synonyms) or *pathogenic stimulus*
Type 1: IL-1β activation disorders (inflammasomopathies)		
Intrinsic		
FCAS[a], MWS[b], NOMID[c]/CINCA[d]	*NLRP3/CIAS1* (1q44)	NLRP3[e] (cryopyrin, NALP3, PYPAF1)
Extrinsic		
FMF[f]	*MEFV* (16p13.3)	Pyrin (marenostrin)
PAPA[g]	*PSTPIP1* (15q24–25.1)	PSTPIP1[h] (CD2BP1[i])
CRMO[j]/SAPHO[k]	Complex	
Majeed syndrome	*LPIN2* (18p11.31)	Lipin-2
HIDS[l]	*MVK* (12q24)	Mevalonate kinase
Recurrent hydatidiform mole	*NLRP7* (19q13)	NLRP7 (NALP7, PYPAF3, NOD12)
DIRA[m]	*IL1RN*	IL-1Ra
Complex/acquired		
Gout, pseudogout	Complex	*Uric acid/CPPD*
Fibrosing disorders	Complex	*Asbestos/silica*
Type 2 diabetes mellitus	Complex	*Hyperglycemia*
Schnitzler syndrome	Sporadic	





FMF: Clinical Features

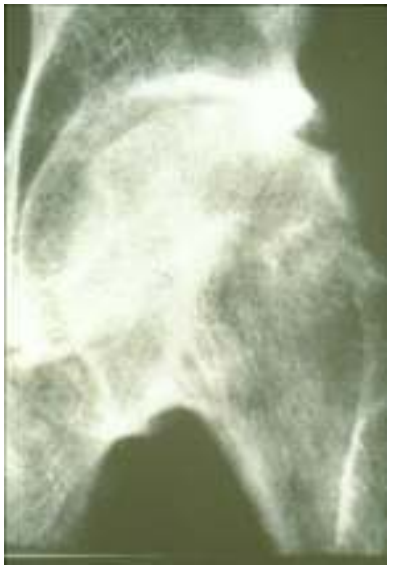
1-3 day episodes of fever with:

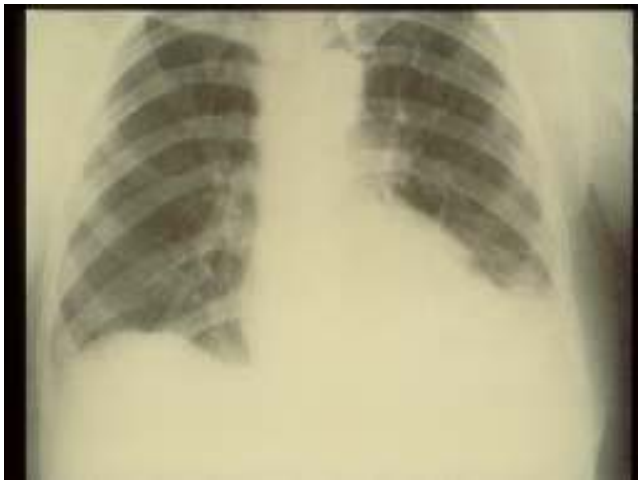
•Abdominal pain

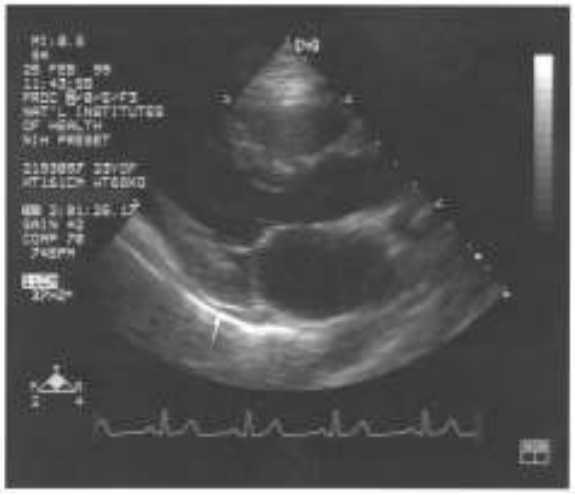
•Chest pain

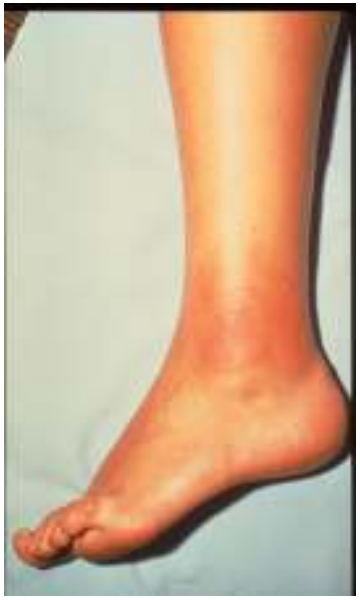
•Arthritis

•Rash



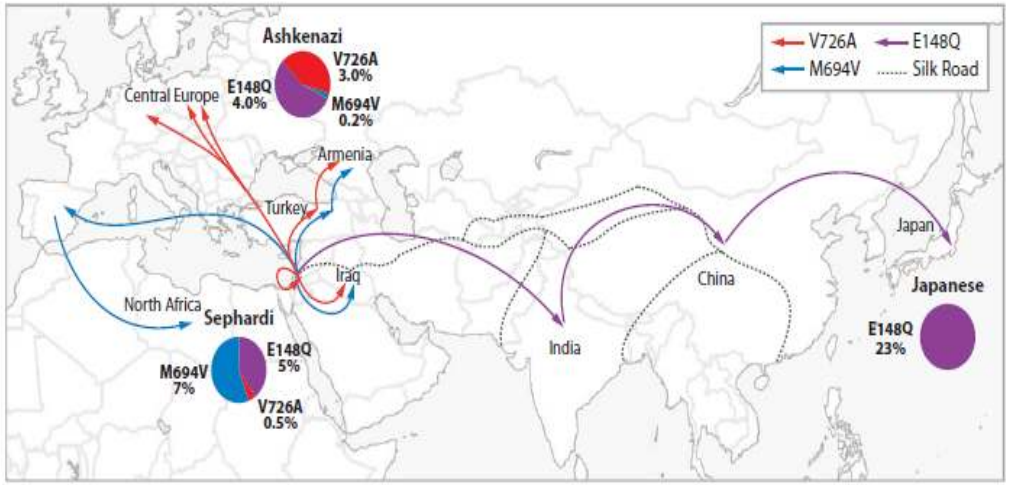






FMF: mutation in the pyrin gene

a



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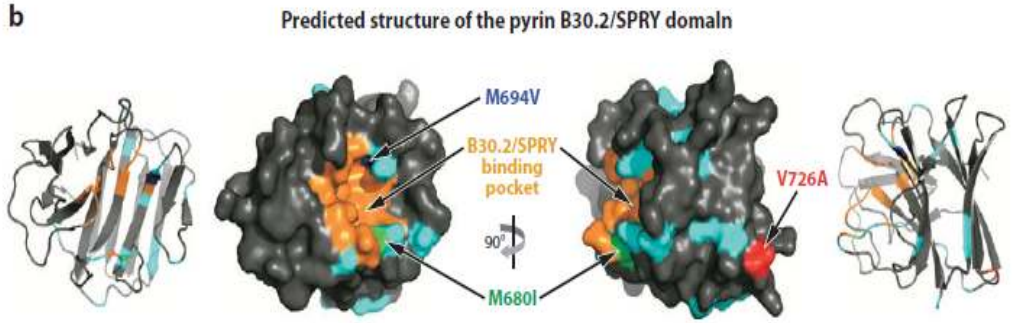
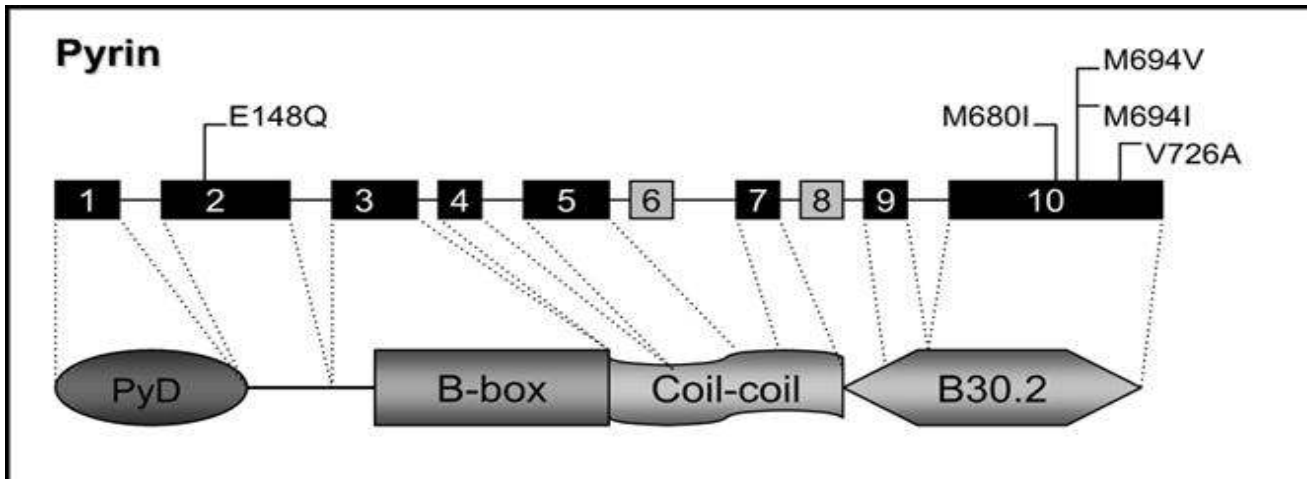


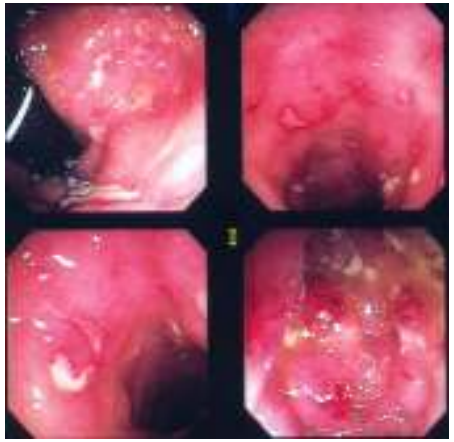
Figure 3

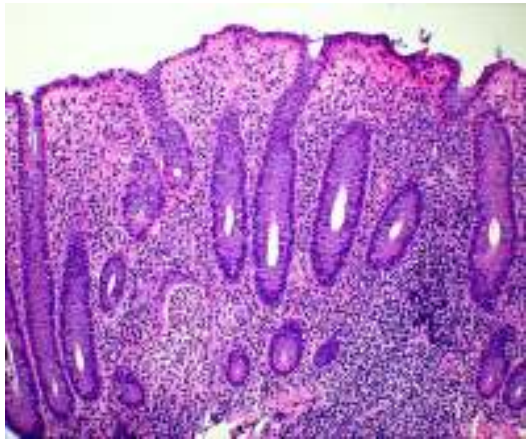
Familial Mediterranean Fever	Mutations of pyrin – an inflammasome inhibitor – may lead to defective down-regulation of inflammasome's activation or to direct activation of caspase-1

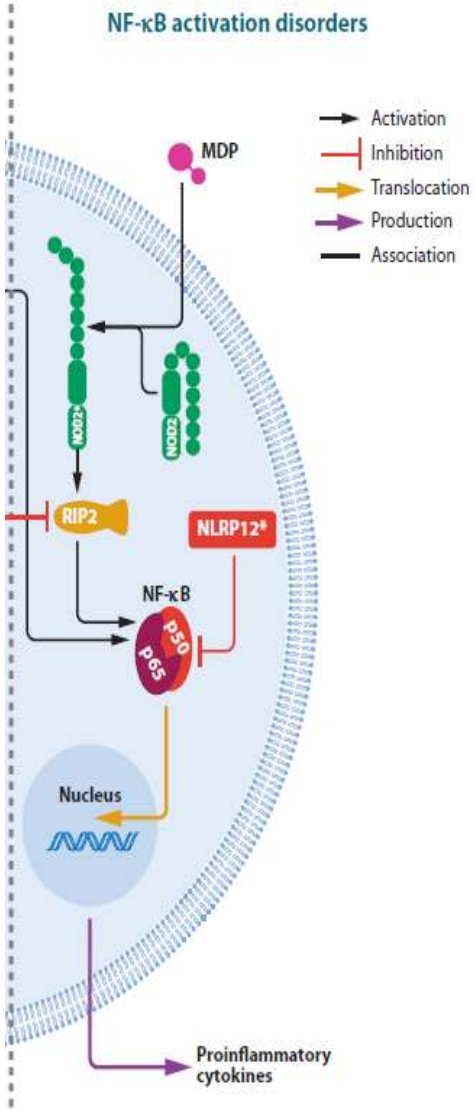


Sidiropoulos et al Ann Rheum Dis 2007

Type 2: NF-κB activation disorders	Gene	Stimulus
Crohn's disease	Complex	*Muramyl dipeptide*
	NOD2 (16p12)	NOD2[n] (CARD15)
	ATG16L1 (2q37.1)	ATG16L1°
	IRGM (5q33.1)	IRGM[p]
Blau syndrome	*NOD2* (16p12)	NOD2 (CARD15)
FCAS2 (Guadaloupe periodic fever)	*NLRP12* (19q13.4)	NLRP12 (NALP12)

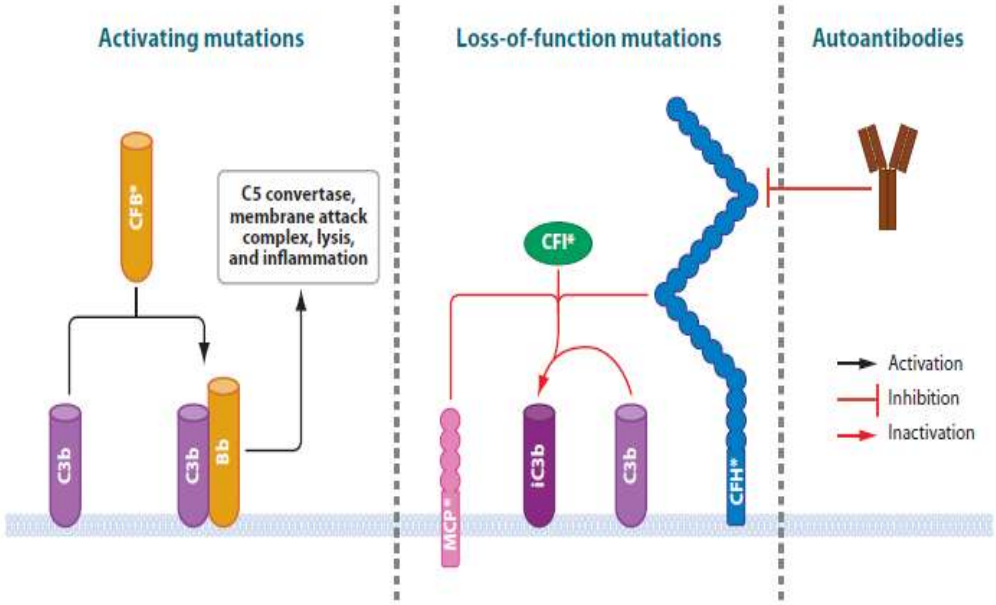




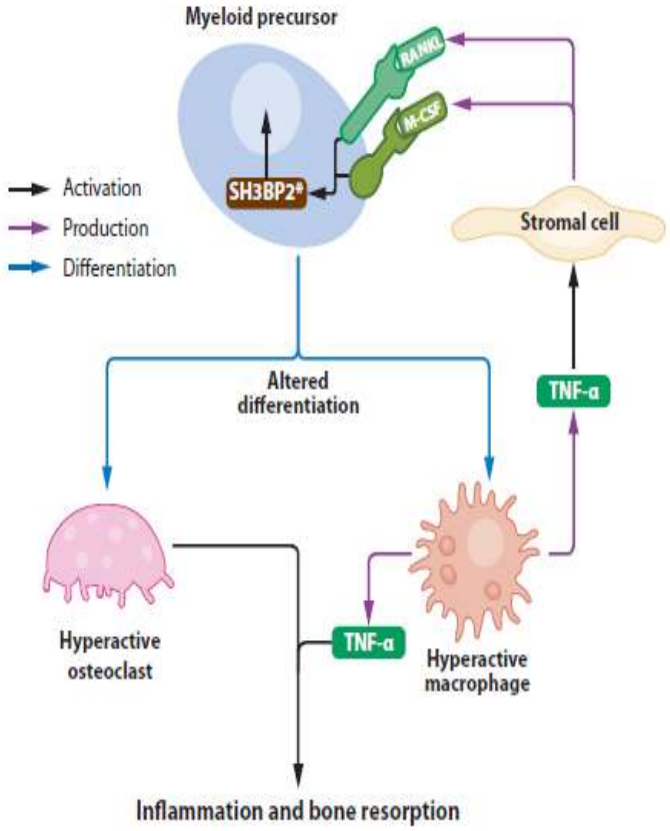


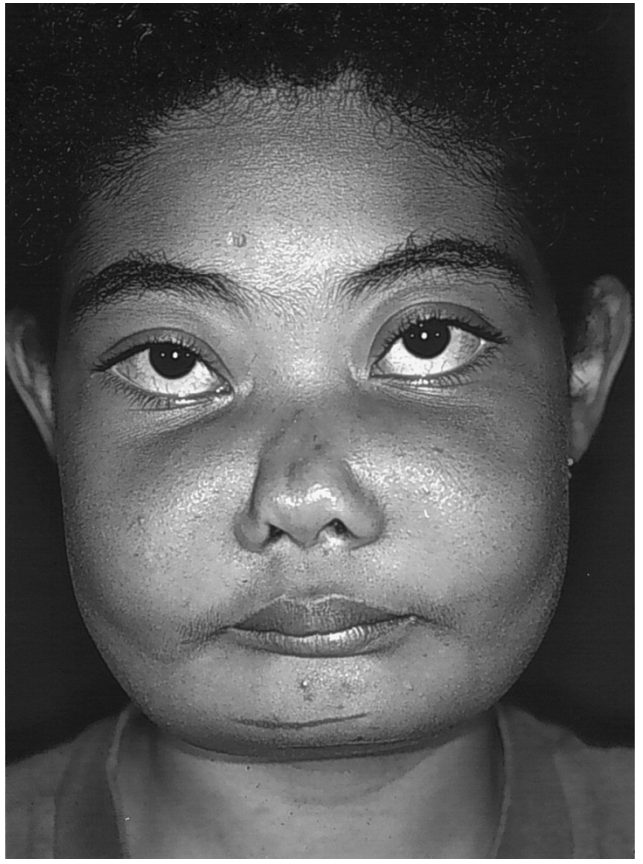
Complement activation syndromes

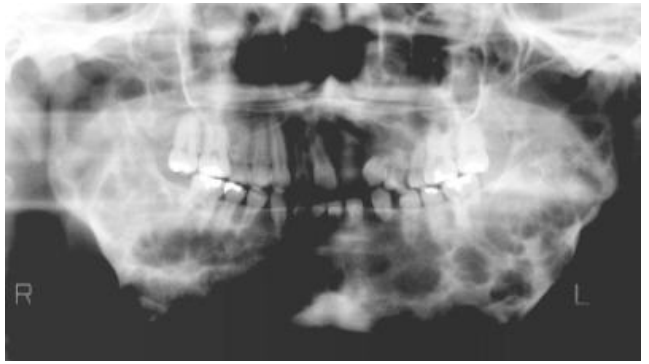
Type 4: Complement disorders		
aHUS[u]	*CFH* (1q32)	Complement factor H
	MCP (1q32)	MCP[v] (CD46)
	CFI (4q25)	Complement factor I
	CFB (6p21.3)	Complement factor B
	Complex	*Autoantibodies*
AMD[w]	Complex	
	CFH (1q32)	Complement factor H



Aberrant cytokine signaling: cherubism

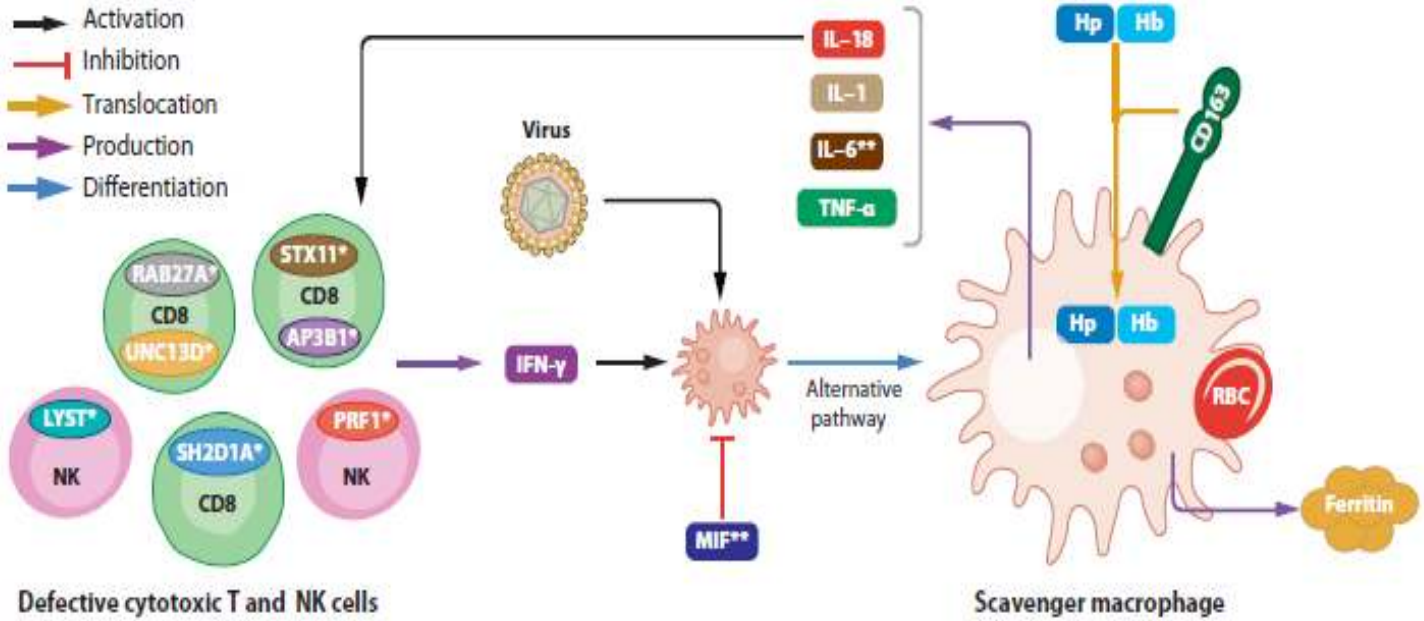






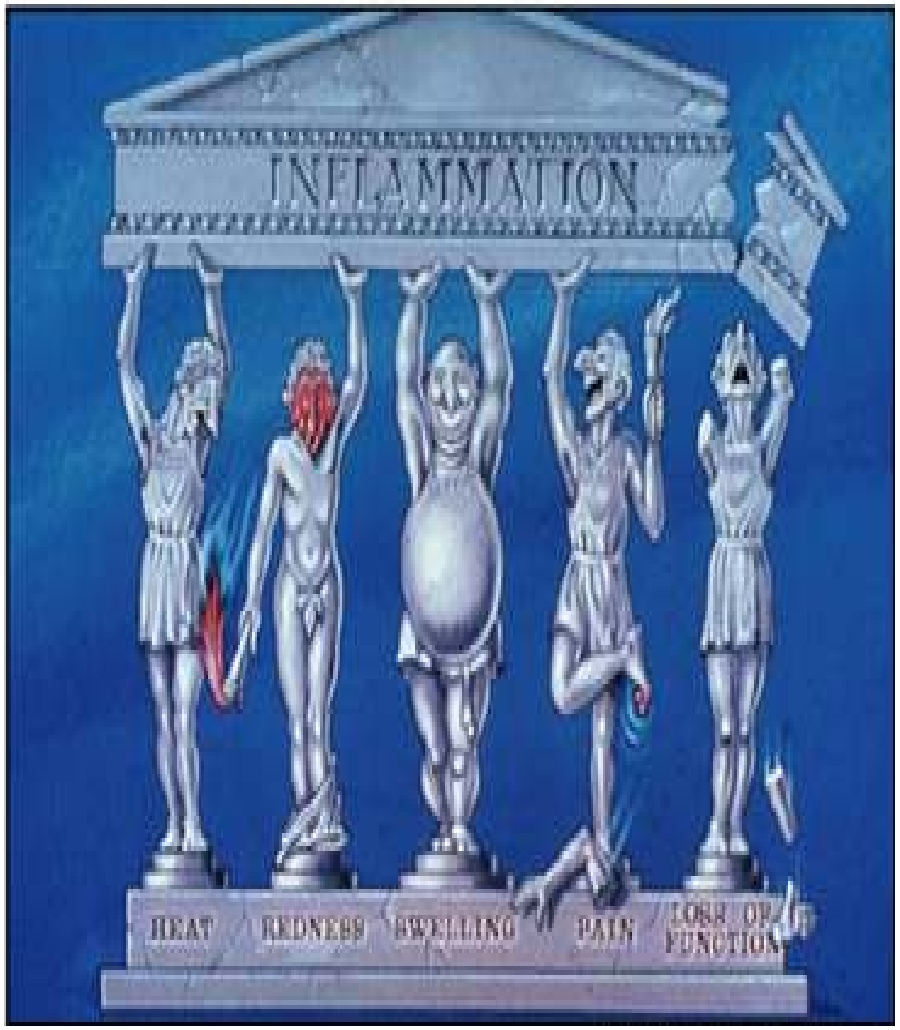
Macrophage activating syndromes: Molecular lesions that affect their activation

Type 6: Macrophage activation		
Familial HLH[x]	*UNC13D* (17q21.1)	Munc13-4
	PRF1 (10q22)	Perforin 1
	STX11 (6q24.2)	Syntaxin 11
	Complex	*Virus*
Chediak-Higashi syndrome	*LYST* (1q42.3)	LYST[y] (CHS1)



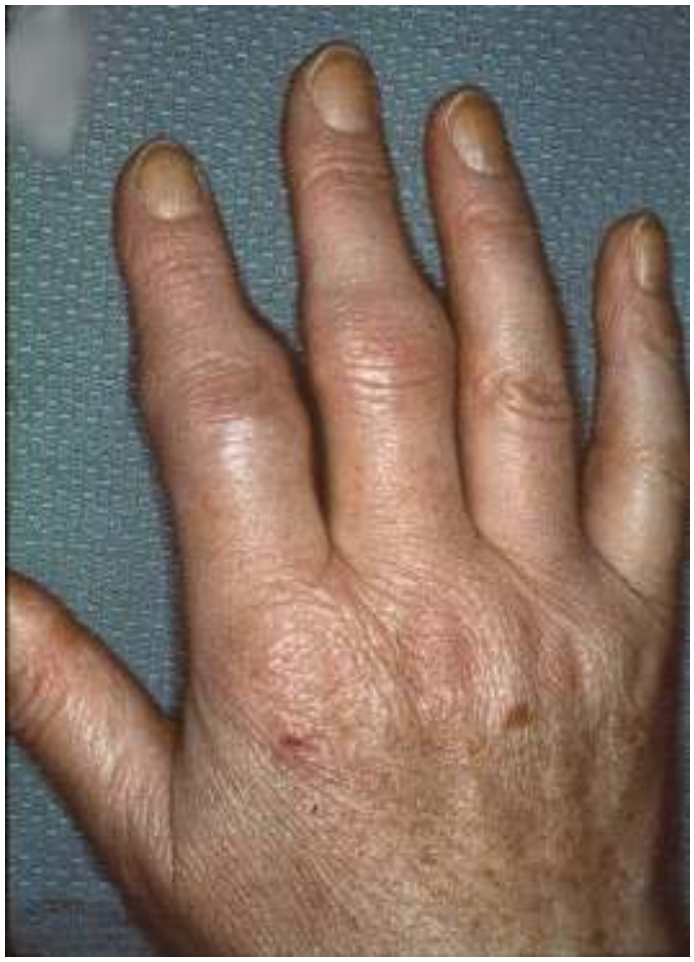
Disease	Gene (chromosome)	Protein (synonyms) or *pathogenic stimulus*
Griscelli syndrome	*RAB27A* (15q21.3)	RAB27A
X-linked lymphoproliferative syndrome	*SH2D1A* (Xq25)	SAP[z]
Hermansky-Pudlak syndrome	*HPS1-8*	HPS1-8[aa]
Secondary HLH	Complex	
Atherosclerosis	Complex	*Cholesterol*

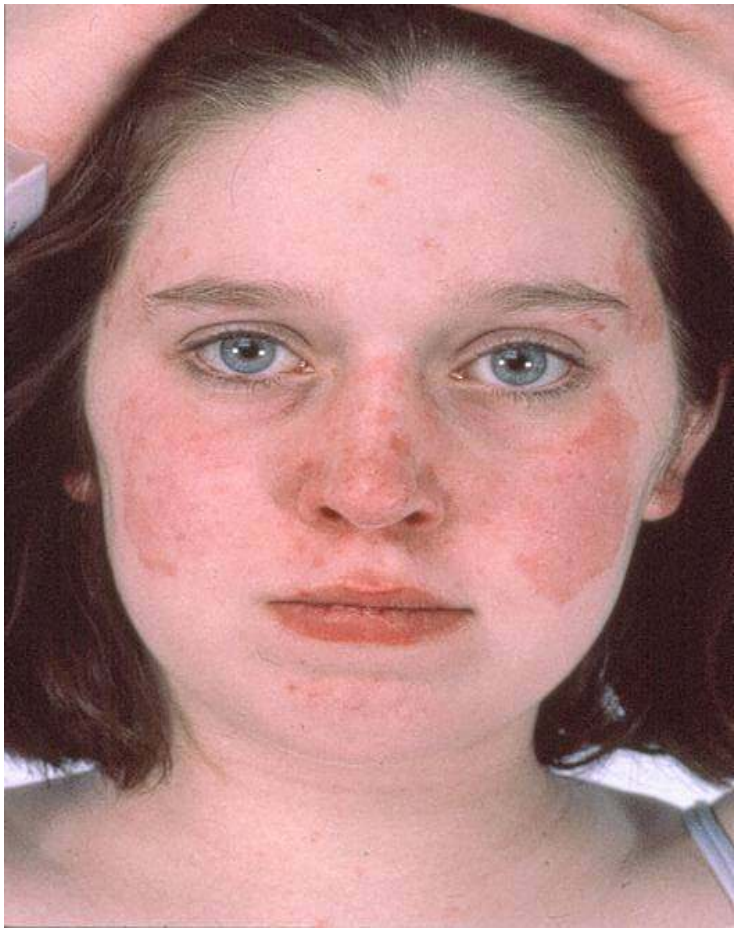
Inflammation



Autoimmunity

Rheumatoid arthritis AND systemic lupus erythematosus





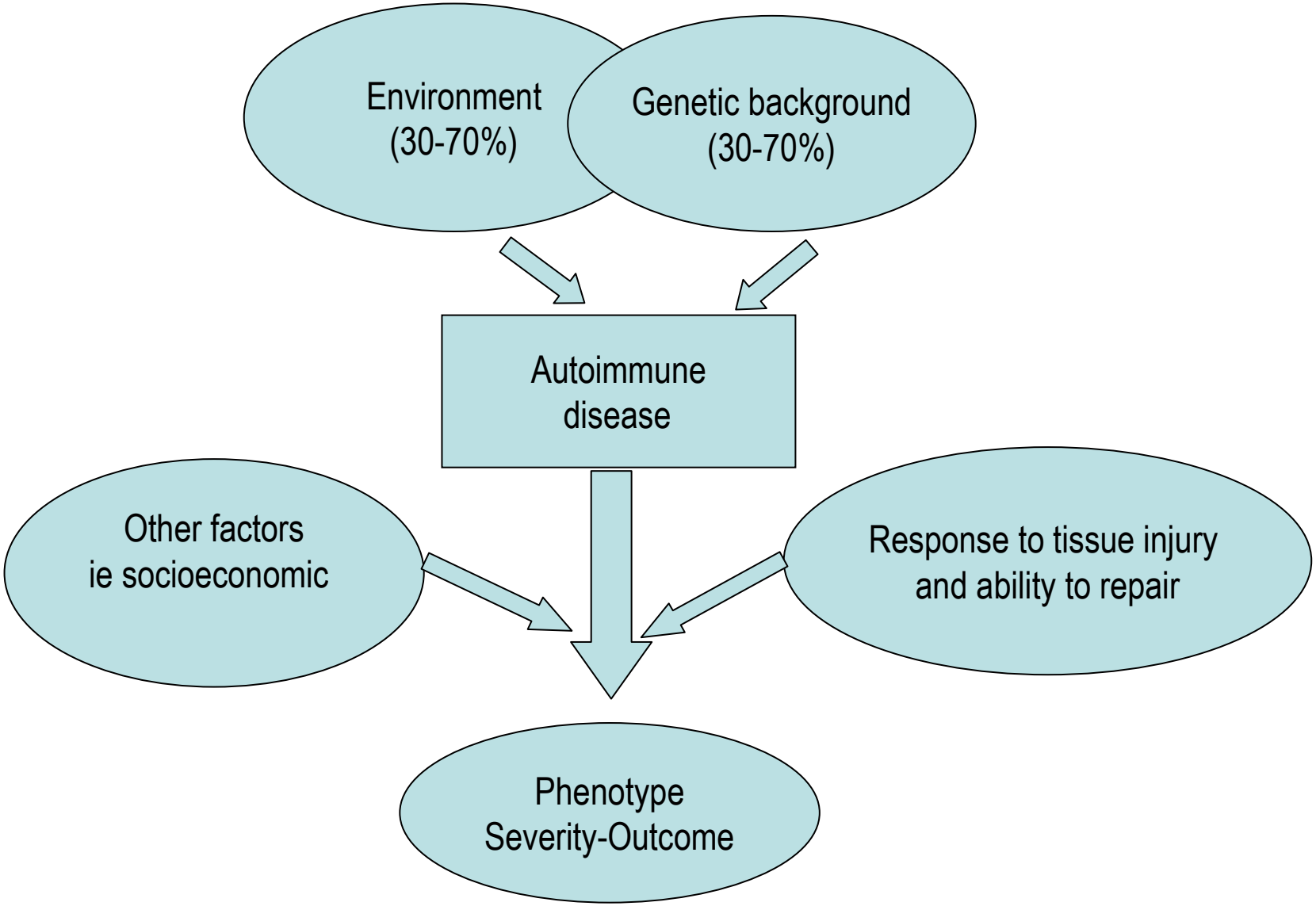
Unique tools to gain insights into inflammation and autoimmunity

Pathogenesis of autoimmune diseases

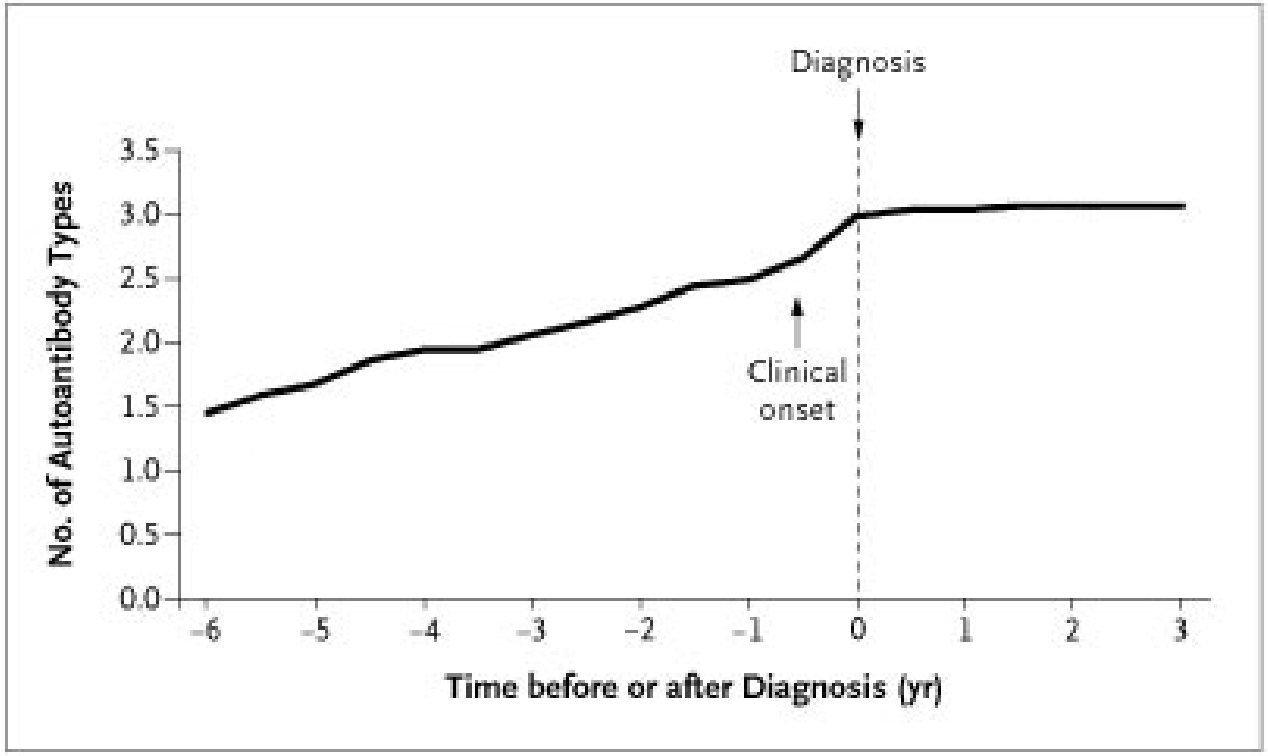
Etiology is unknown but the pathogenetic mechanisms are well delineated

Complex Pathogenesis in autoimmune/inflammatory diseases

Etiology is unknown but the pathogenetic mechanisms are well delineated



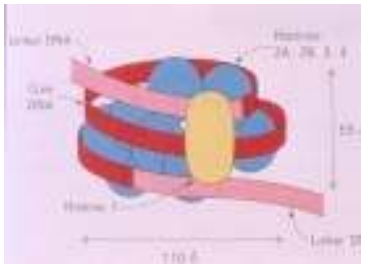
Accumulation of Systemic Lupus Erythematosus Autoantibodies

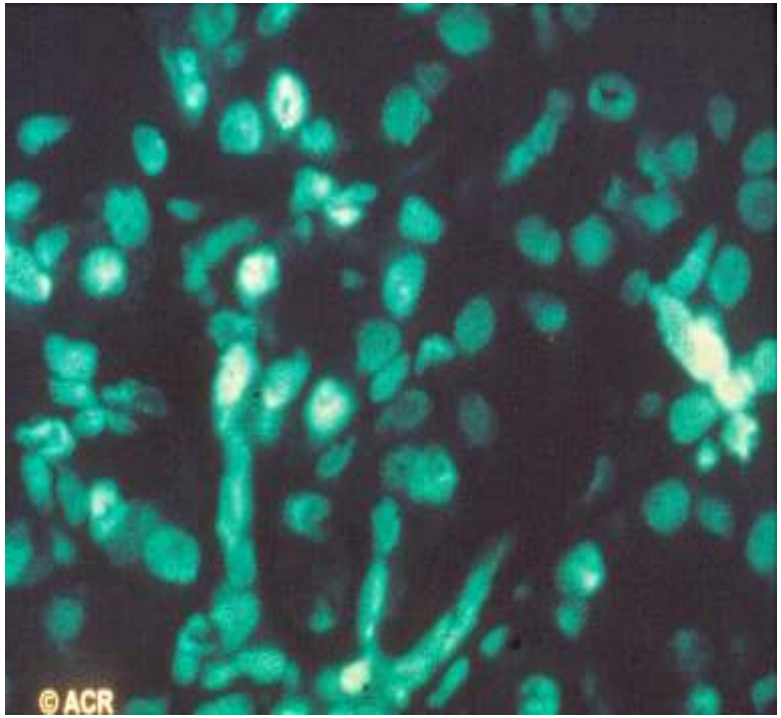


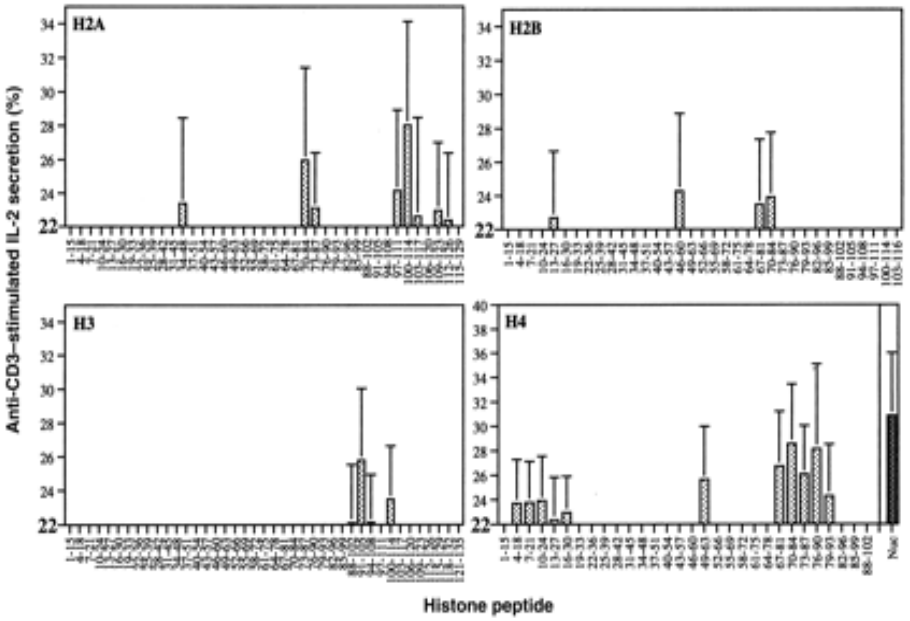
Autoimmunity

- **Low level of auto-reactivity is crucial for the normal function of the immune system**
- ***Autoimmunity:*** **adaptive immunity against self-constituents (auto-antibodies and auto-reactive T cells)**
- ***Autoimmune disease*****: adaptive immunity against self-constituents in the absence of infection or other discernible cause resulting in tissue injury or dysfunction**
- **Autoimmunity although in some cases can be liked to inflammation represents a distinct process**

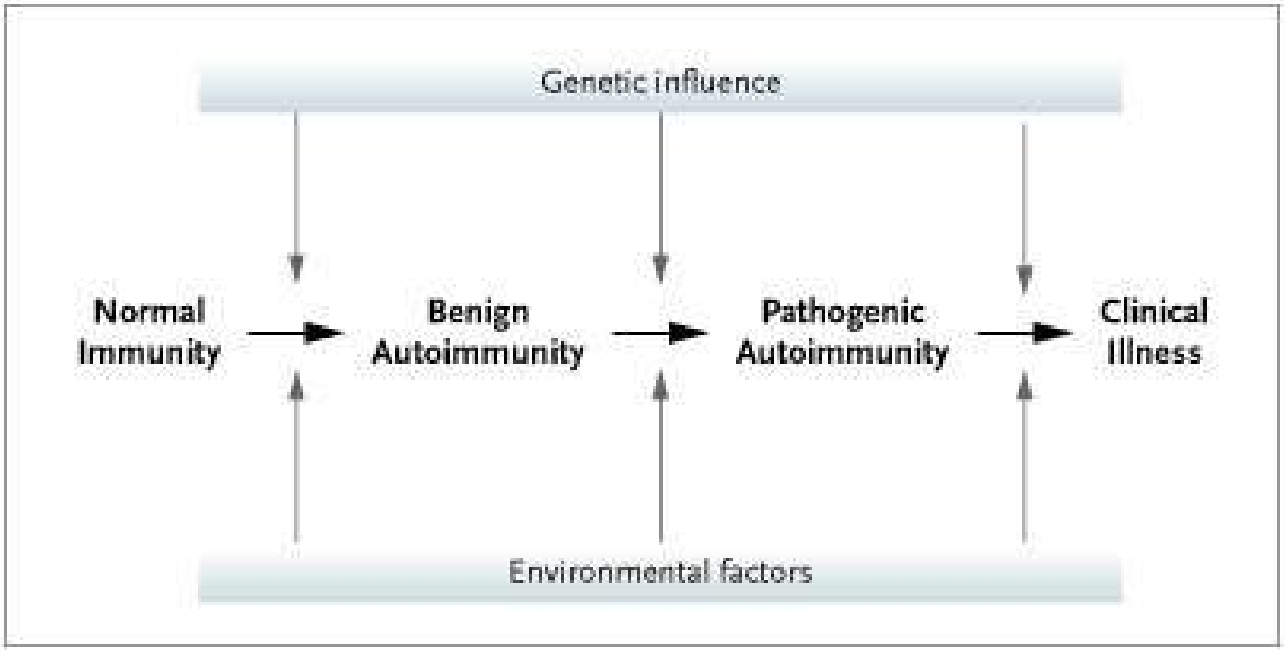
Antinuclear antibodies (ANA) and T cell responses vs histonic peptides in SLE



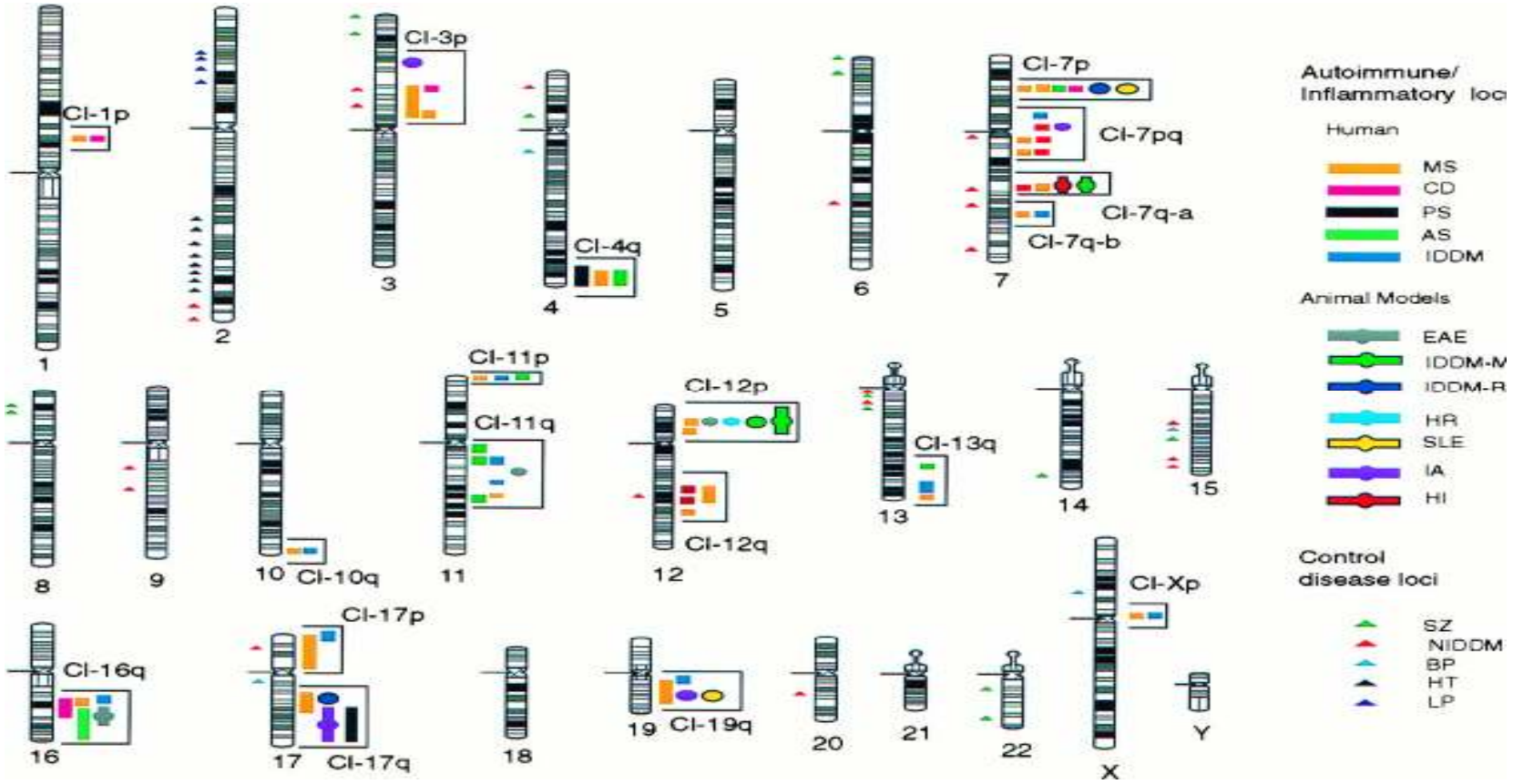




Phases in the Development of Pathogenic Autoimmunity



Multiple loci in man and in mice; some common across a variety of diseases



Becker KG et al. Proc Natl Acad Sci U S A 1998;Aug 18:95

Confirmed genetic associations in AD

Intracellular signaling and receptors

Intracellular pattern recognition receptors

Cytokines and receptors

Autophagy related

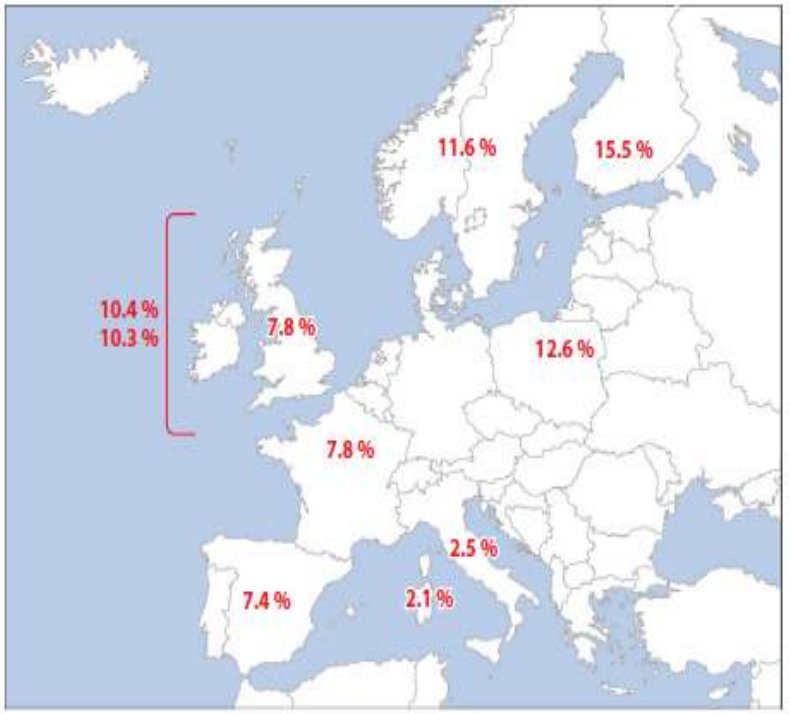
Table 1 Genetic loci with confirmed associations with human autoimmune disorders

Gene	Location	Function	Diseases[a]
Intracellular signaling molecules and receptors			
PTPN22	1p13.3	TCR and BCR signaling and other?	RA, SLE, AITD, T1D
BANK1	4q22	B cell activation/BCR signaling	SLE
TNFAIP3	6q23	Ubiquitin editing enzyme; inhibitor of TNFR signaling/NF-κB pathway	RA, SLE, CD
BLK	8p23	B cell activation	SLE
PTPN2	8p11.3	Negative regulator of T cell activation	CD, T1D
TRAF1	9q33	Regulates TNFR signaling/NF-κB pathway	RA
Intracellular pattern-recognition receptors			
IFIH1	2q24	Receptor for viral dsRNA	T1D, GD
NOD2/CARD15	16q12	Intracellular receptor for bacteria, signals via NF-κB	CD
Transcription factors			
REL	2p13	Member of NF-κB	RA
STAT4	2q32.2	Regulates IFN-γ pathway	RA, SLE
IRF5	7q32	Regulates type 1 IFN pathway	SLE
NKX2-3	10q24.2	Regulates development of intestinal and secondary lymphoid organs and B and T cell homing	CD
Cytokines and cytokine receptors			
IL2/Il21	4q26	T cell regulation	T1D, RA, Celiac disease
IL23R	1p31.1	Th17 homeostasis	PSA, PSO, CD, AS
IL7RA	5p13	Memory T cell homeostasis	MS
IL2RA	10p15.1	T cell/Treg homeostasis	MS, T1D, GD
IL12B	15q31.1	Development of T cell subsets, Th1 and Th17	PSO, CD
Membrane receptors and costimulatory molecules			
CTLA4	2q33	T cell costimulation inhibitory	T1D, RA
ITGAM	16p11.2	Immune complex clearance/leukocyte adhesion	SLE
CD40	20q12	B/T cell costimulation	RA
		Production of IgM, TNF-α, IL-2 via NF-κB pathway	
Autophagy related			
ATG16L1	2q37.1	Autophagy	CD
IRGM	5q33.1	Autophagy	CD
Enzymes			
ARTS1	5q15	Peptide trimming for MHC I	AS
PADI4	1p36.13	Enzymatic peptide citrullination	RA
Autoantigens			
INS	11p15.5	Target autoantigen	T1D
TSHR	14q31	Target autoantigen	AITD

GWAS in autoimmune diseases: general themes

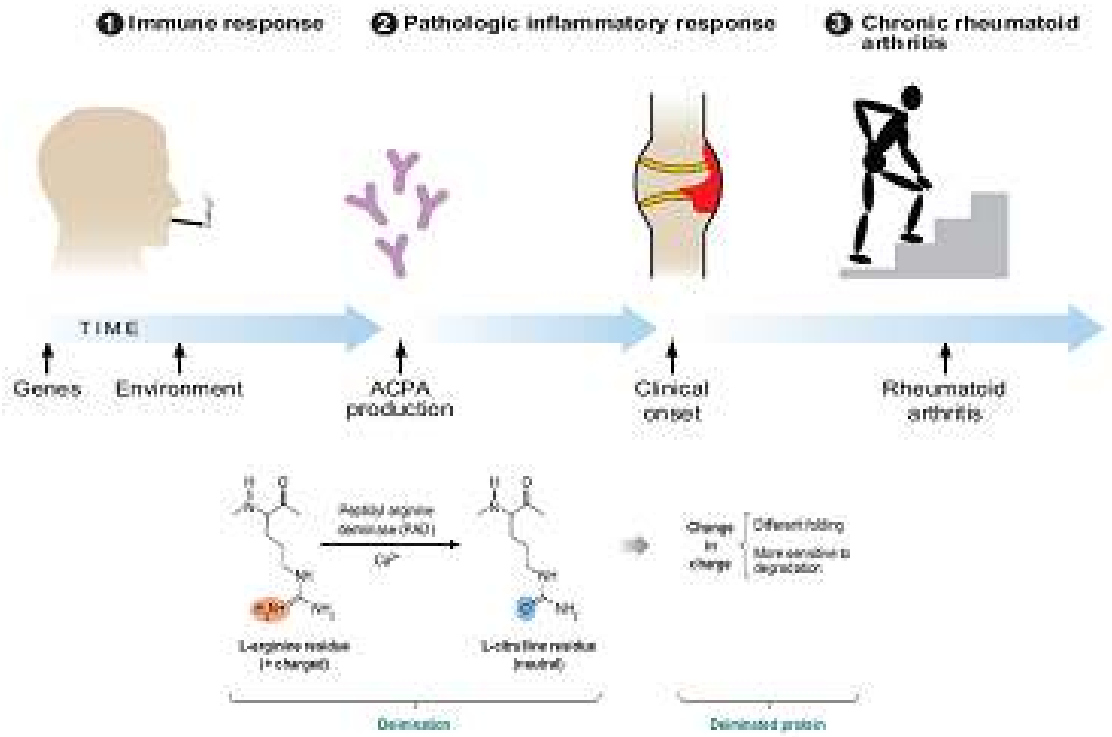
- **Autoimmune disorders have a complex genetic basis**
- **Multiple genes contribute to disease risk, each with generally modest effects independently.**
- **Common genes underlie multiple autoimmune disorders.**
- **Heterogeneity among subphenotypes within a disease and across major racial groups.**
- **The current crop of genetic associations are only the start of a complete catalog of genetic factors forautoimmunity**
- **It remains unclear to what extent common variation versus multiple rare variants contribute to disease susceptibility**.

PTPN2 risk alleles in Europe



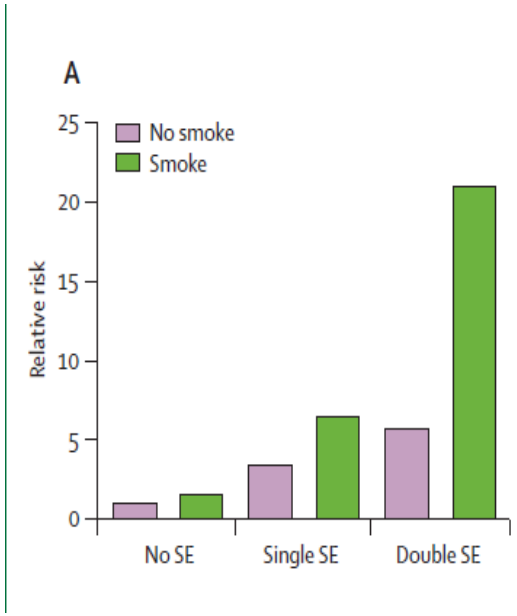
P Gregersen Annu. Rev. Immunol. 2009. 27:363–91

Interplay between genes and environment in RA



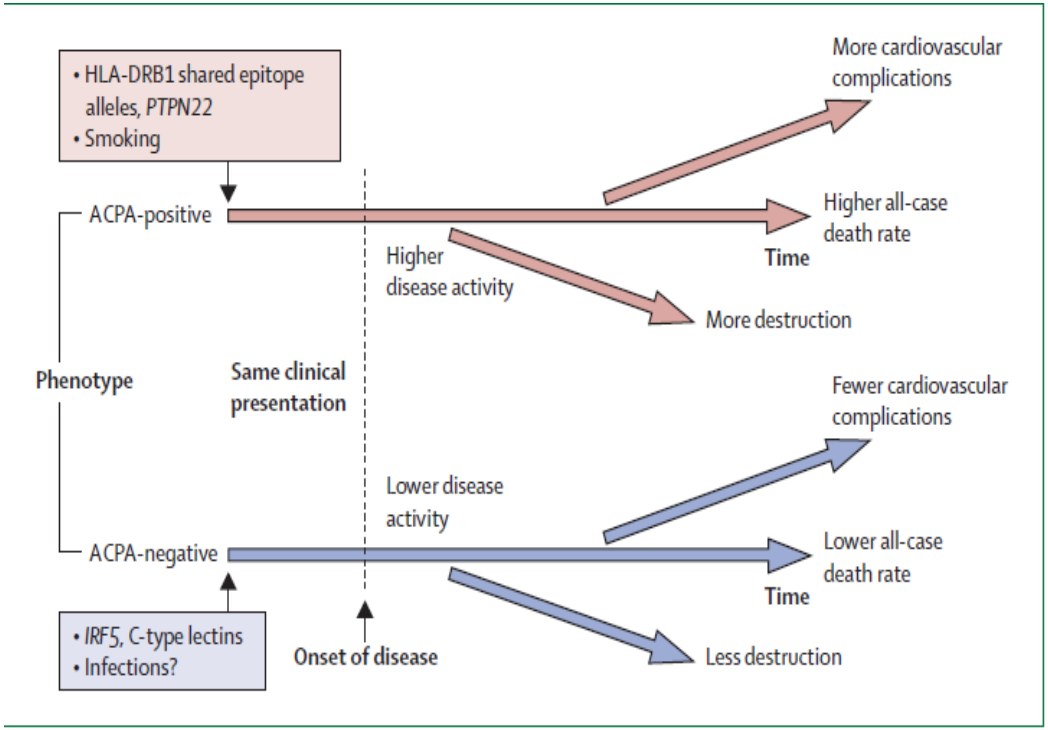
Smoking causes citrullination of proteins in the lungs and the joints:
antibodies to citrulinated proteins appear in patients with certain HLA-haplotypes several years before the disease

In genetically susceptible patients with the shared epitope (HLA-DR4) smoking increases significantly the risk but only in patients with anti-CCP antibodies



Same phenotype (albeit more severe diseases) but different subset of the disease as defined by serology and genetics

Difference in risk factors, immune response and disease outcomes in subsets of RA



Similar phenotype

Putting everything together genes, enviroment and immune inflammatory response in RA

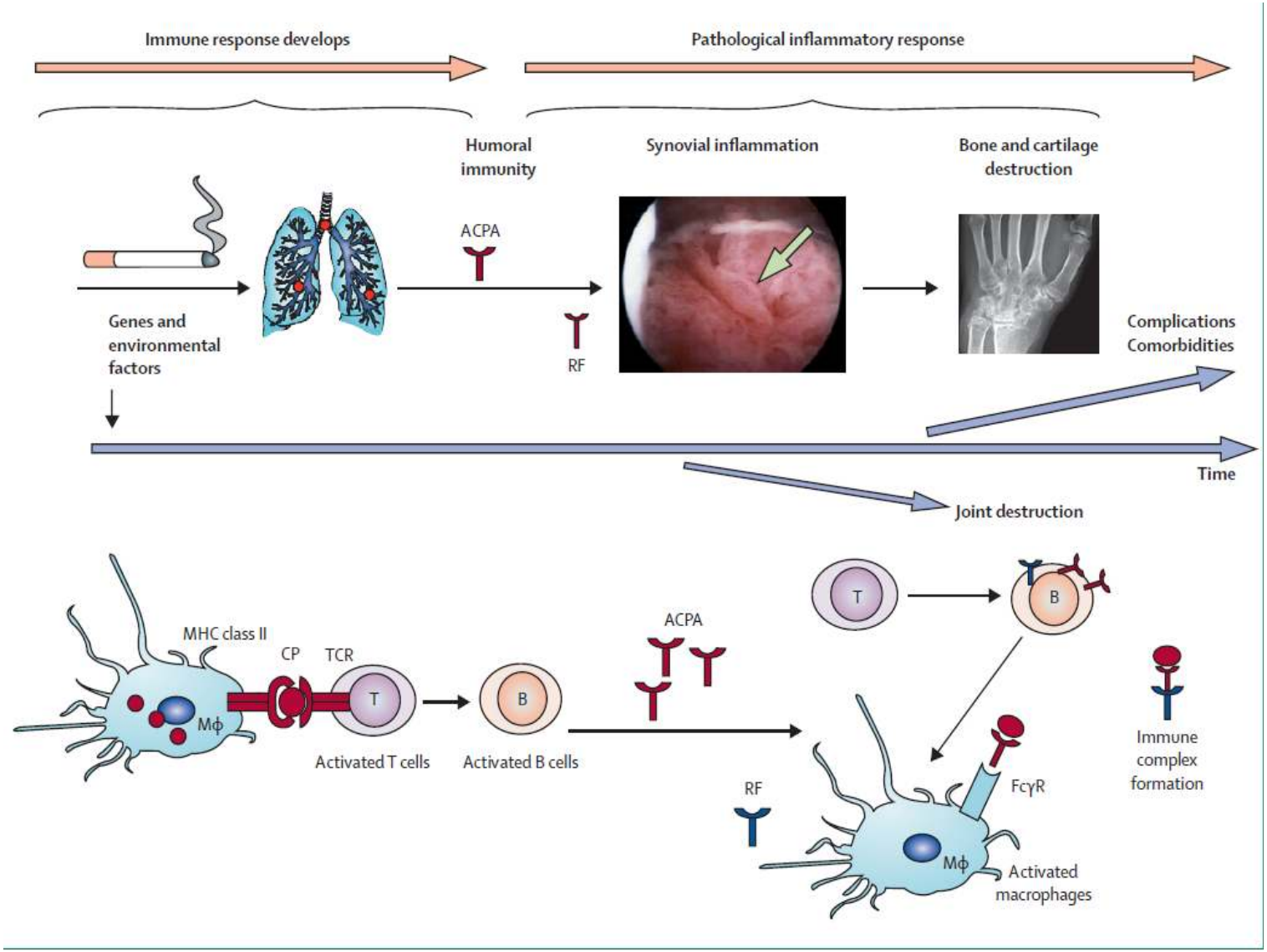
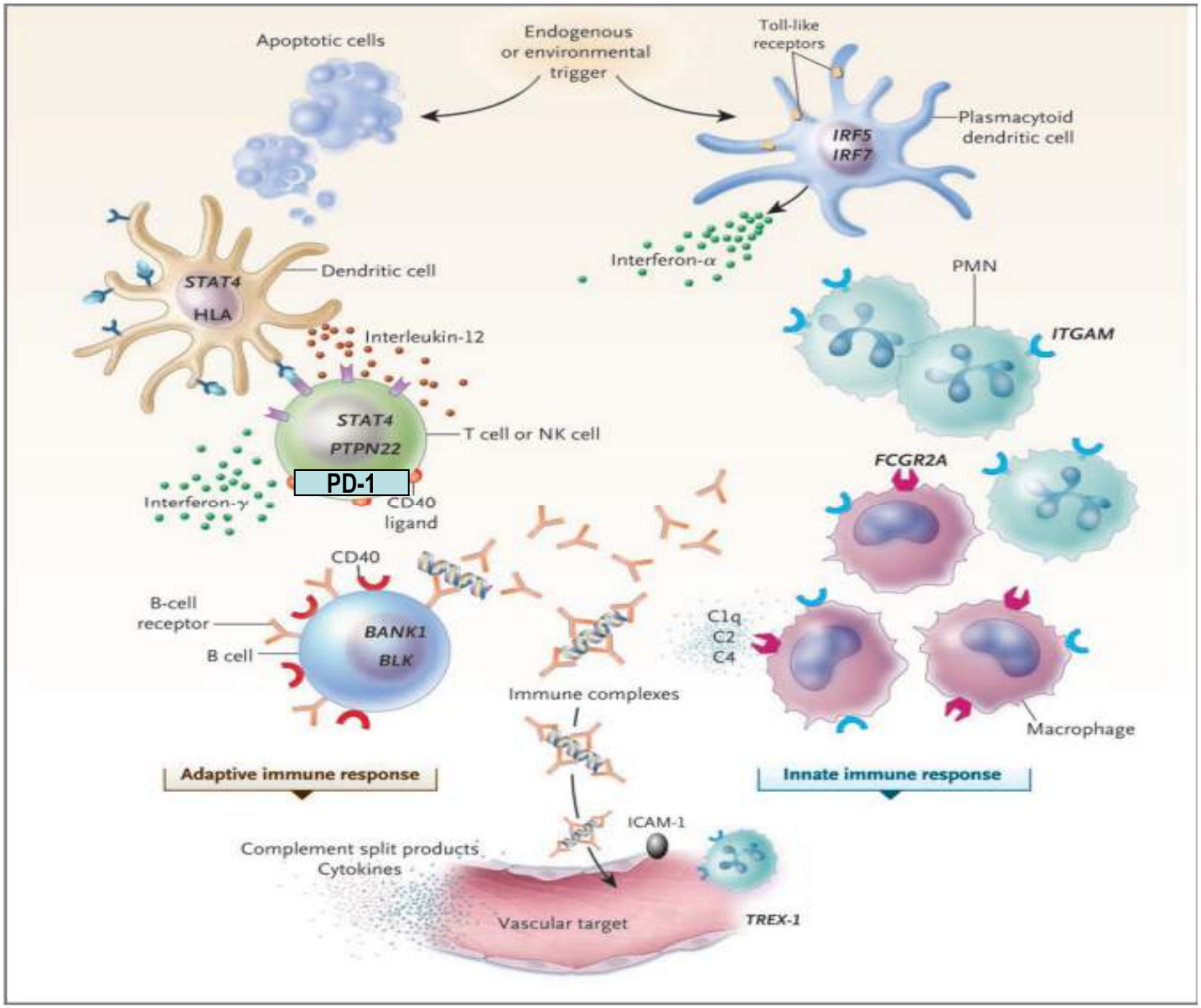


Table 1. New and Confirmed Genetic Variants Conferring a Significant Risk of Systemic Lupus Erythematosus in Two Genomewide Association Studies.*

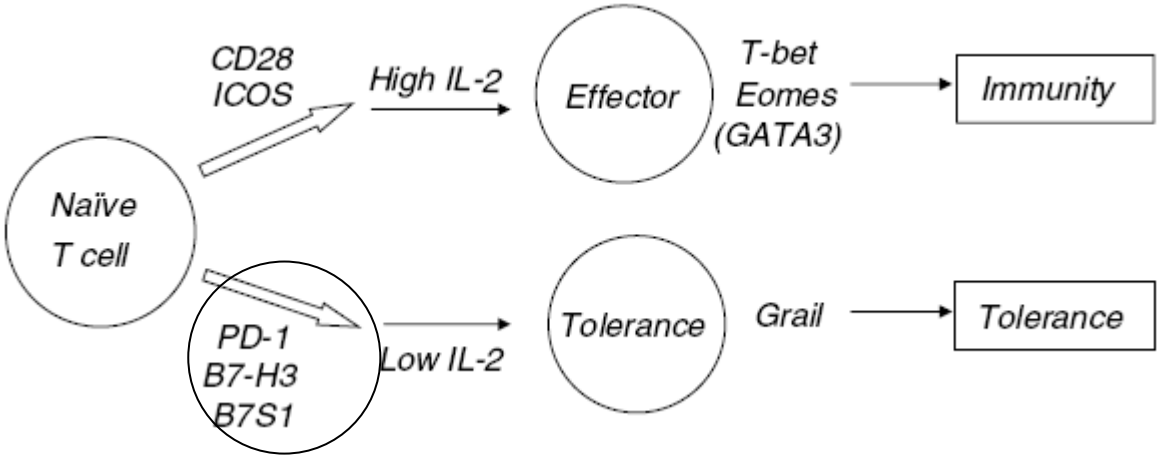
Gene	Genome Location	Proposed Function
HLA†	6p21.33	Presentation of antigen
HLA‡	6p21.32	Presentation of antigen
ITGAM‡	16p11.2	Adhesion of leukocytes to endothelial cells
IRF5‡	7q32.1	Production of interferon-α
KIAA1542†	11p15.5	Linkage disequilibrium with *IRF7*; production of type I interferon
PXK†	3p14.3	Unknown effect of serine–threonine kinase
PTPN22†	1p13	Inhibition of lymphocyte activation
FCGR2A†	1q23	Clearance of immune complexes
STAT4‡	2q32	Modulation of the production of cytokines in T cells and natural killer cells; activation of response of macrophages to interferon-α
BLK§	8p23.1	Activation of B cells

Genes , enviroment and immune response (innate and adaptive) in SLE



Functional genetics in human SLE

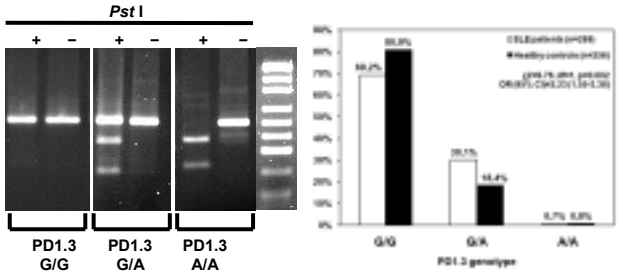
The role of PD-1 in regulation of T cell tolerance in systemic lupus erythematosus



T-cell tolerance or effector function depends on the balance between co-stimulatory and inhibitory signals

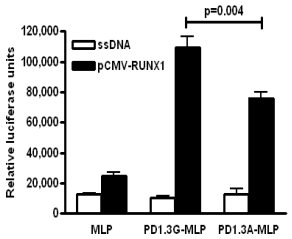
Defective expression and function of PD-1 in human SLE:

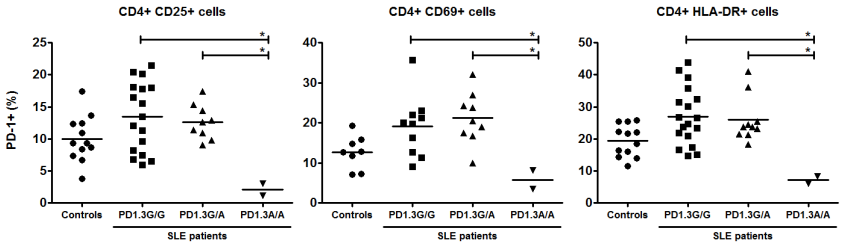
Increased frequency of the regulatory PD1.3A SNP in SLE patients



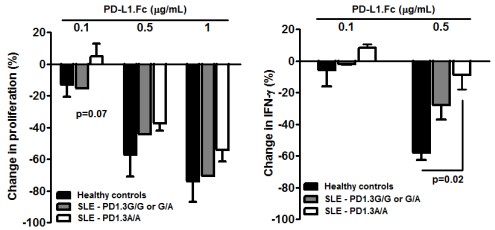
Disruption of a RUNX1-binding site in PD1 gene

Decreased PD-1 expression in presence of PD1.3A SNP





Defective PD-1–mediated suppression of T cells



SLE patients have defective induction of PD-1 in an in vitro model of auto-reactivity

Bertsias et al. Arthritis Rheum 2009

Immune system and systemic autoimmunity

- **During the last 50 years emphasis on adaptive immunity (auto-antibodies, T cells)**
- **In recent years several observations have increased the interest on the innate immunity and its role on the pathogenesis of autoimmunity**
- **Genetic studies have shown the involvement of genes of innate immunity such as interferon regulatory factor 5 (TLR signaling) and NALP1**
- **Increased interferon a in SLE**
- **Complement deficiencies may predispose to SLE**
- **The realization that the production of inflammatory cytokines including IFN-a may be mediated by endogenous ligands such as immune complexes**

Modern genetics and ancient defenses in autoimmunity

ORIGINAL ARTICLE

NALP1 in Vitiligo-Associated Multiple Autoimmune Disease

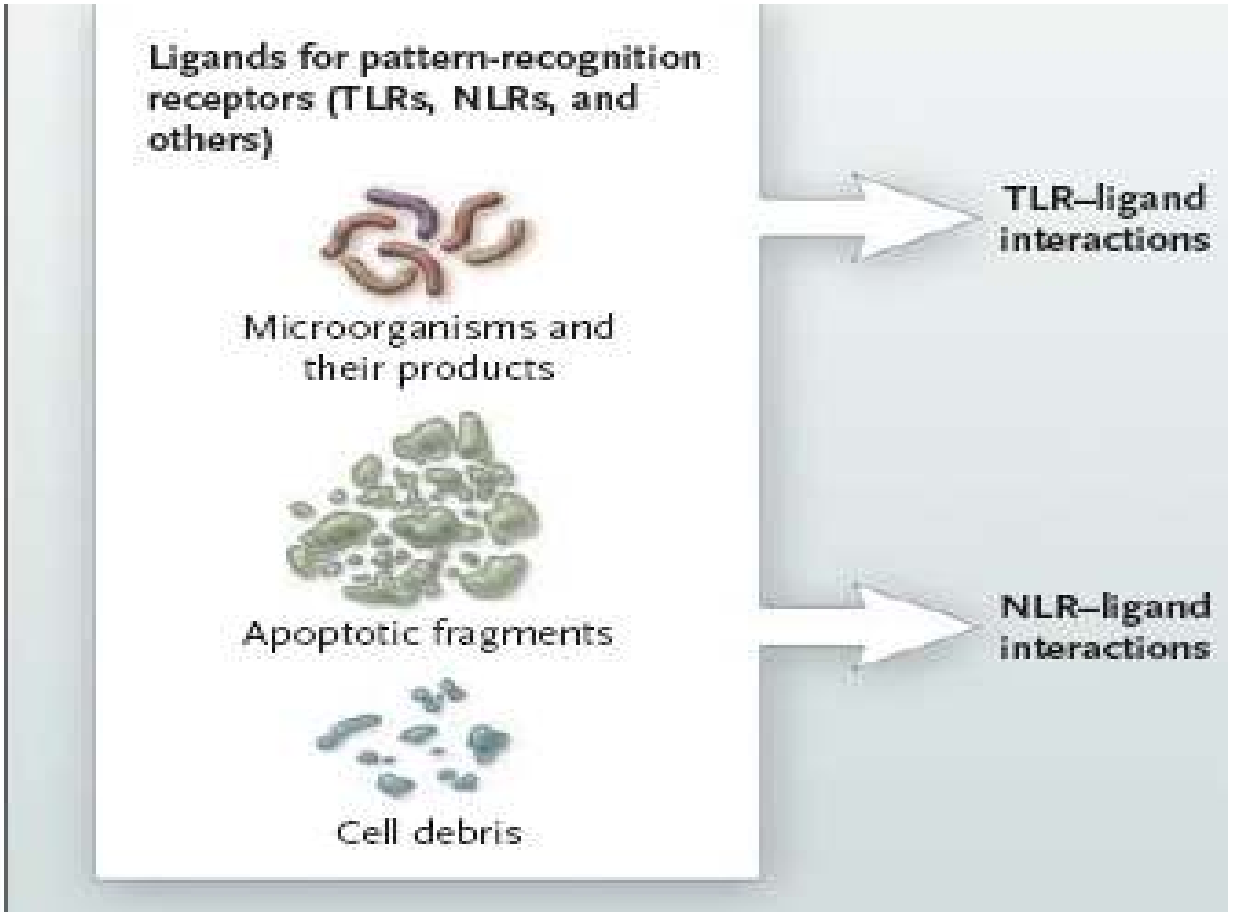
N Engl J Med 2007;356:1216-25.

ized vitiligo, autoimmune thyroid disease, latent autoimmune diabetes in adults, rheumatoid arthritis, psoriasis, pernicious anemia, systemic lupus erythematosus, and Addison's disease.

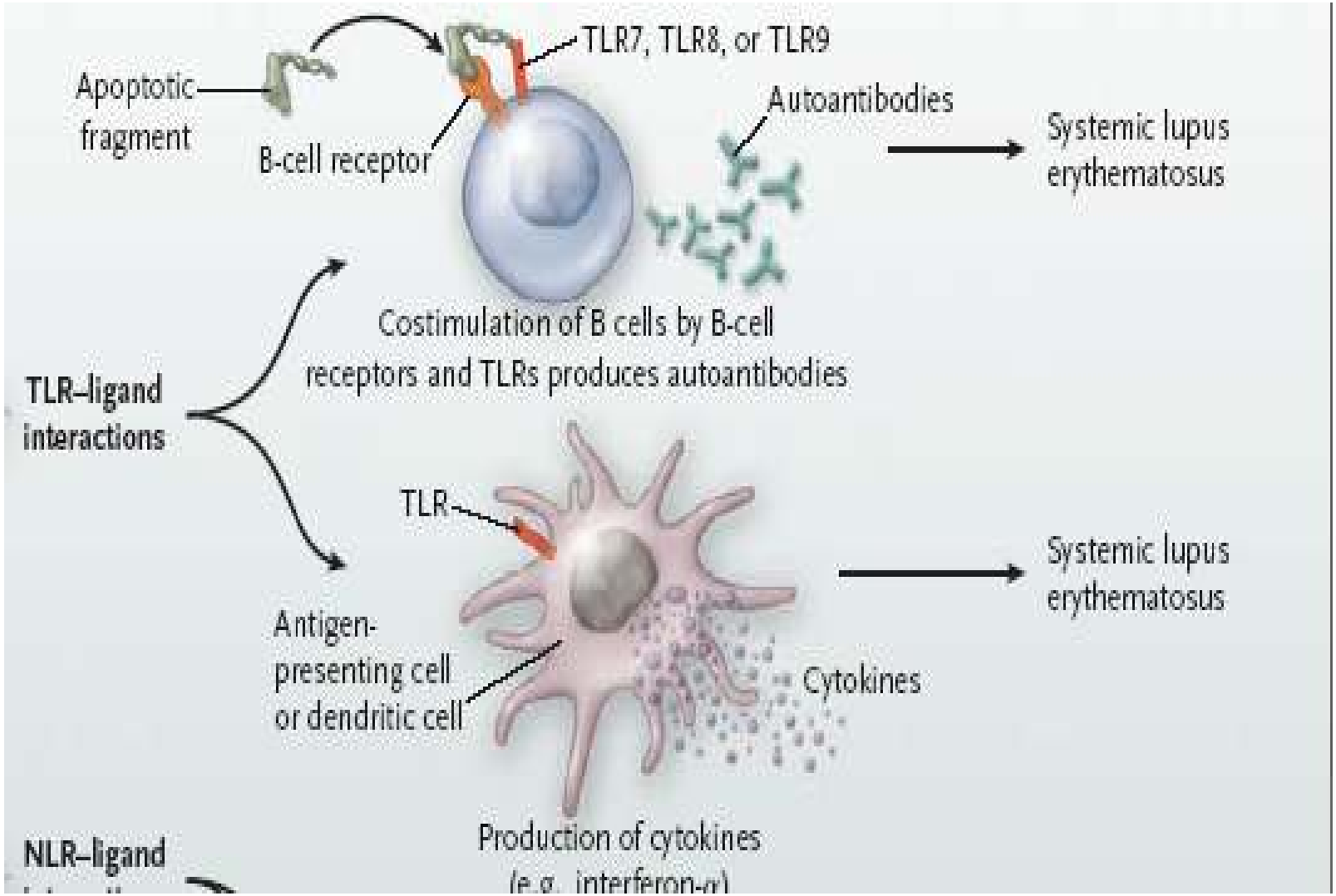
CONCLUSIONS

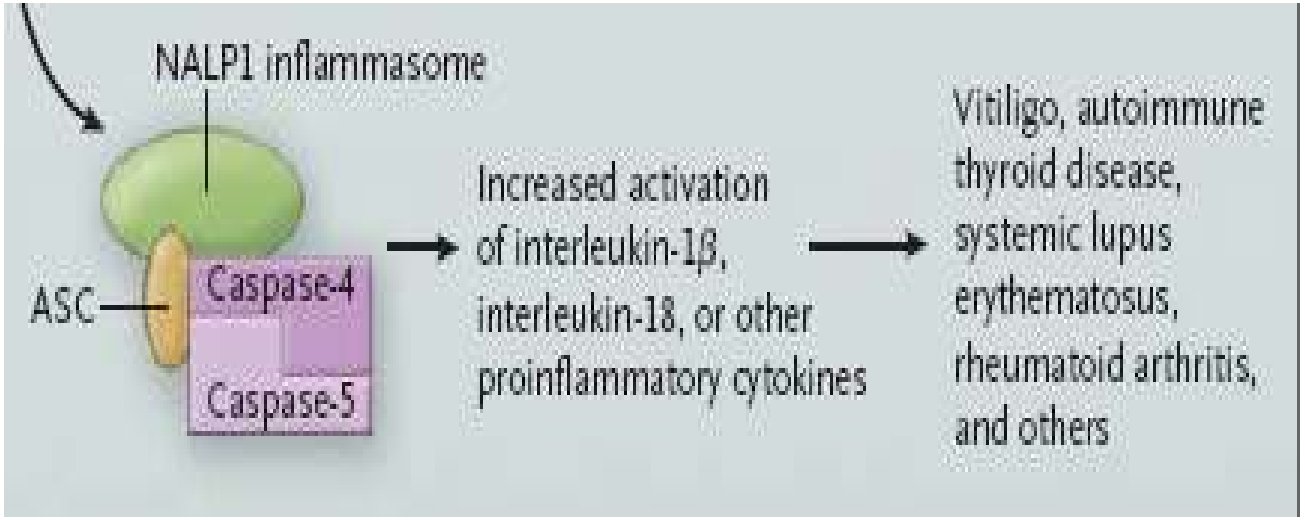
DNA sequence variants in the *NALP1* region are associated with the risk of several epidemiologically associated autoimmune and autoinflammatory diseases, implicating the innate immune system in the pathogenesis of these disorders.

The innate immune system contains several major families of damage associated molecular pattern-recognition receptors(TLRs and NLRs).



DAMPs (Microbial or cellular ligands for TLRs) can costimulate B cells to produce autoantibodies as well as stimulate the production of type 1 interferons that have been found to be dysregulated in SLE




NALP1 inflammasome
ASC
Caspase-4
Caspase-5
Increased activation of interleukin-1β, interleukin-18, or other proinflammatory cytokines
Vitiligo, autoimmune thyroid disease, systemic lupus erythematosus, rheumatoid arthritis, and others

Immunity, Inflammation and Autoimmunity in Humans

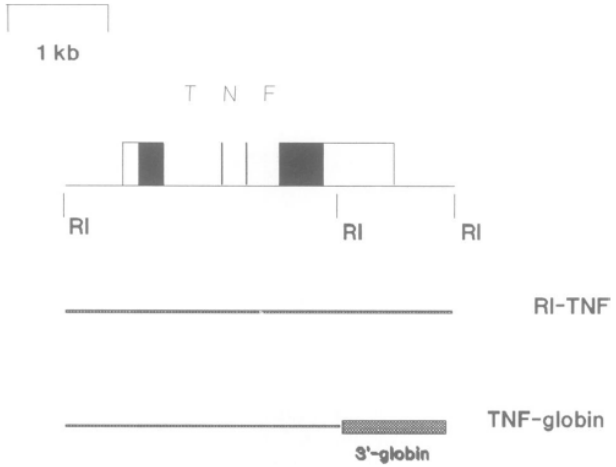
Physiology, pathophysiology, nosology and therapeutics

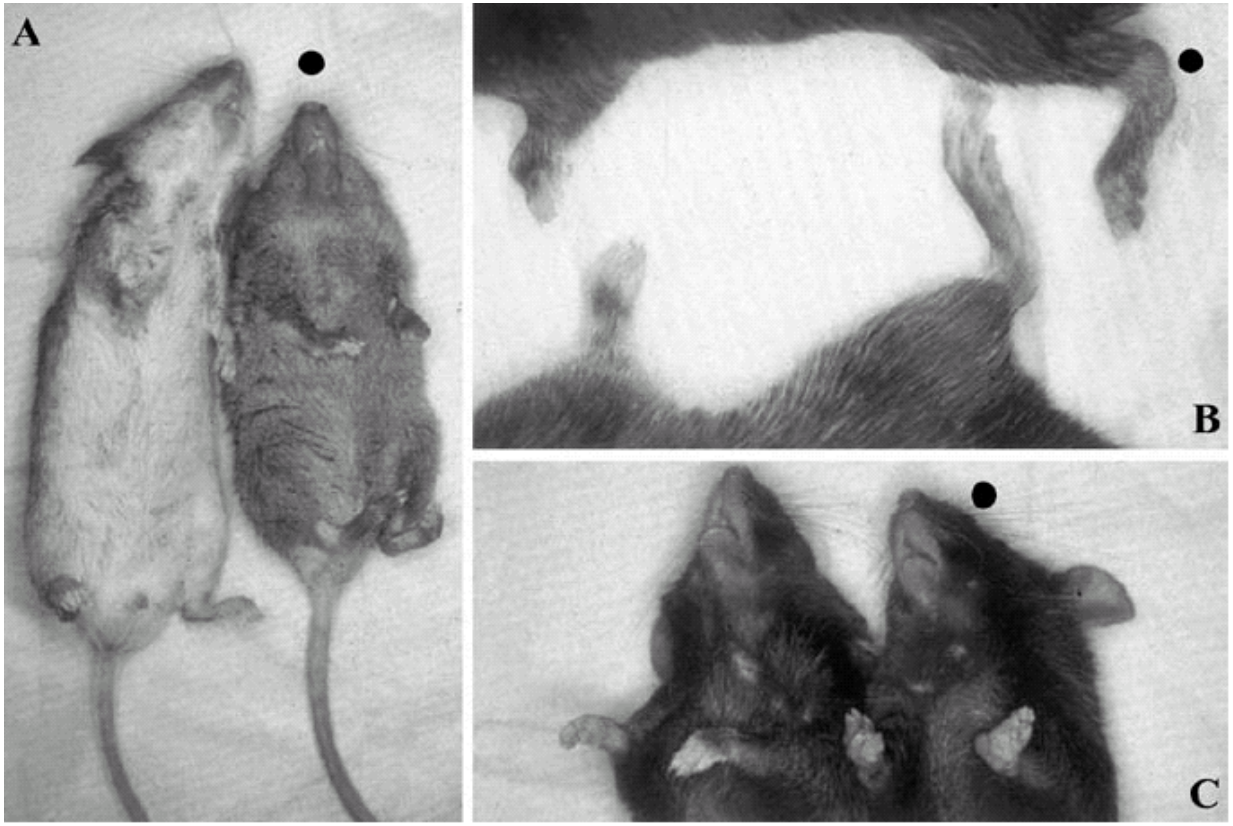
- Origin and physiologic role of inflammation
- *Exogenous* inflammation vs *endogenous* inflammation and associated diseases
- *Endogenous inflammation*: Auto-inflammation vs autoimmune inflammation
  - Auto-inflammatory diseases: Diseases of innate immunity
  - Autoimmune diseases: Diseases of innate and adaptive immunity
- **Biologic therapies: Lessons learned about the targeting of key molecules and cells**
- **Inflammatory and autoimmune diseases are complex: the use of high-throughput methods in their investigation**
- **Perspective**

The EMBO Journal vol.10 no.13 pp.4025–4031, 1991

Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis

Jeanne Keffer, Lesley Probert, Haris Cazlaris, Spiros Georgopoulos, Evangelos Kaslaris[1], Dimitris Kioussis[2] and George Kollias

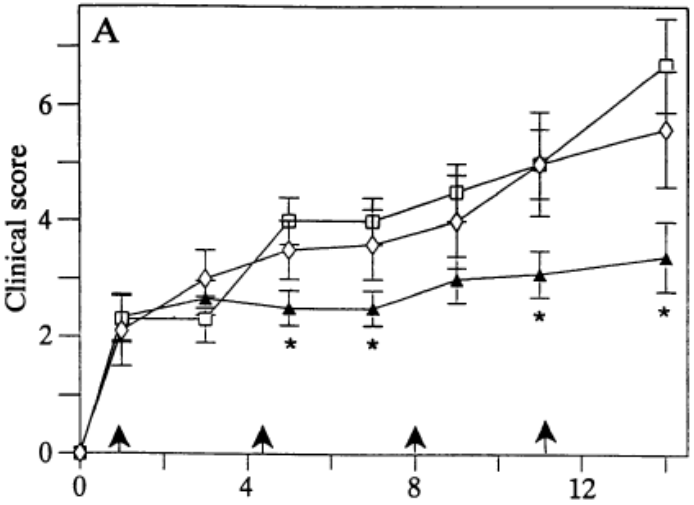


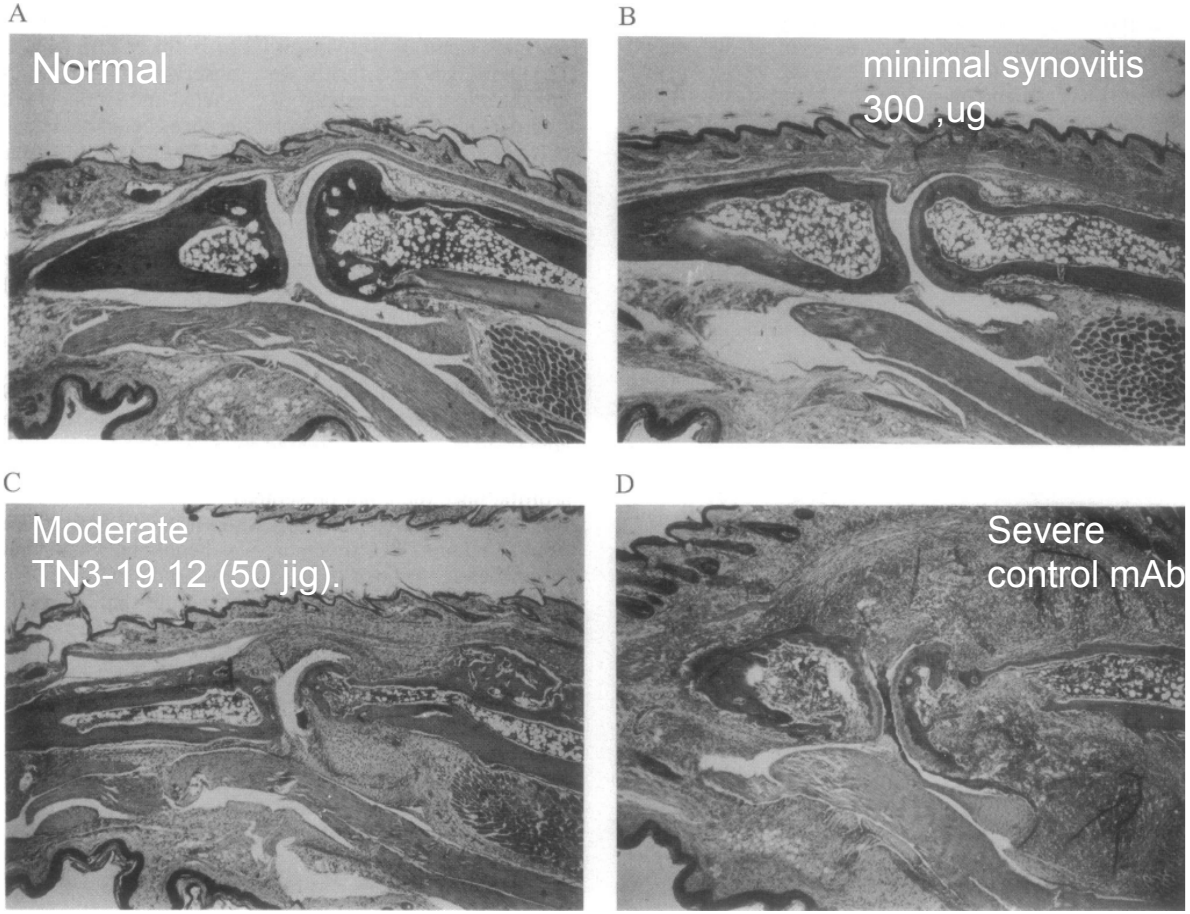


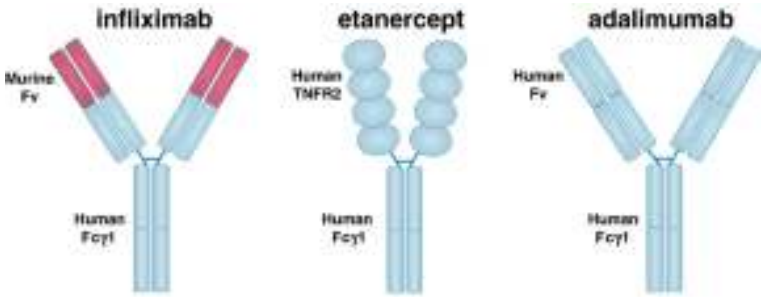
Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 9784–9788, October 1992
Immunology

Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis

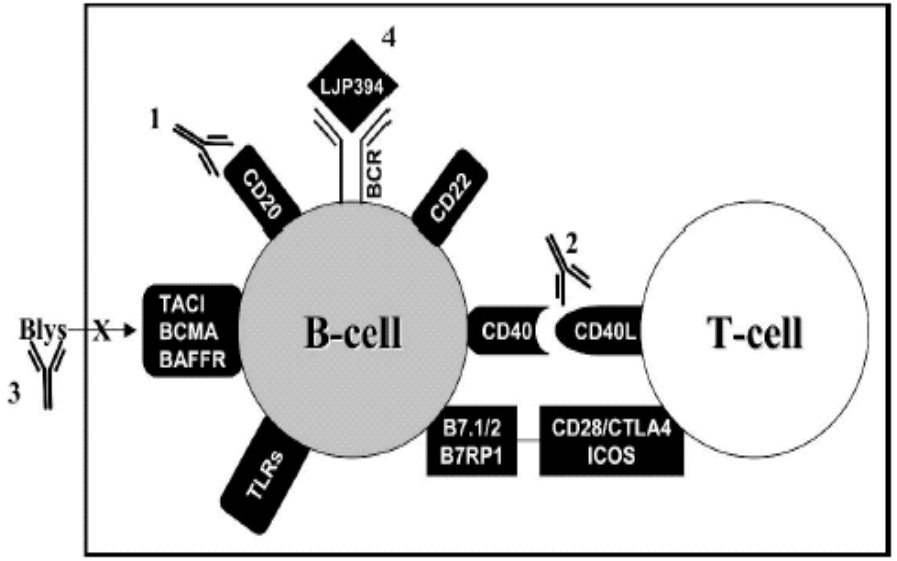
RICHARD O. WILLIAMS*, MARC FELDMANN, AND RAVINDER N. MAINI

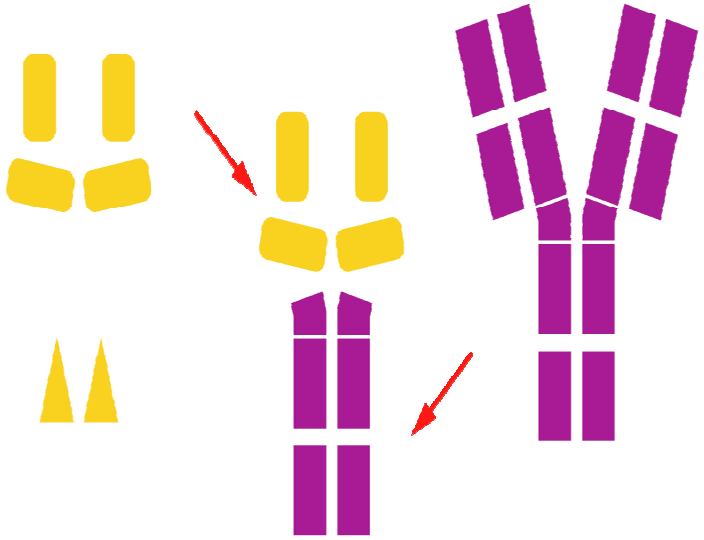






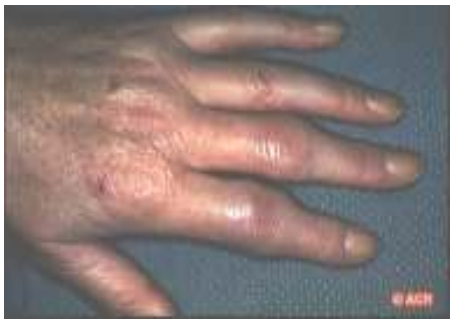
Anti-cytokine
Anti-TNF; anti-IL-6; IL-Ra; anti-p40
anti-IL-17

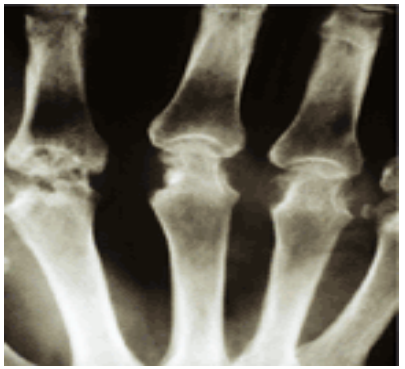


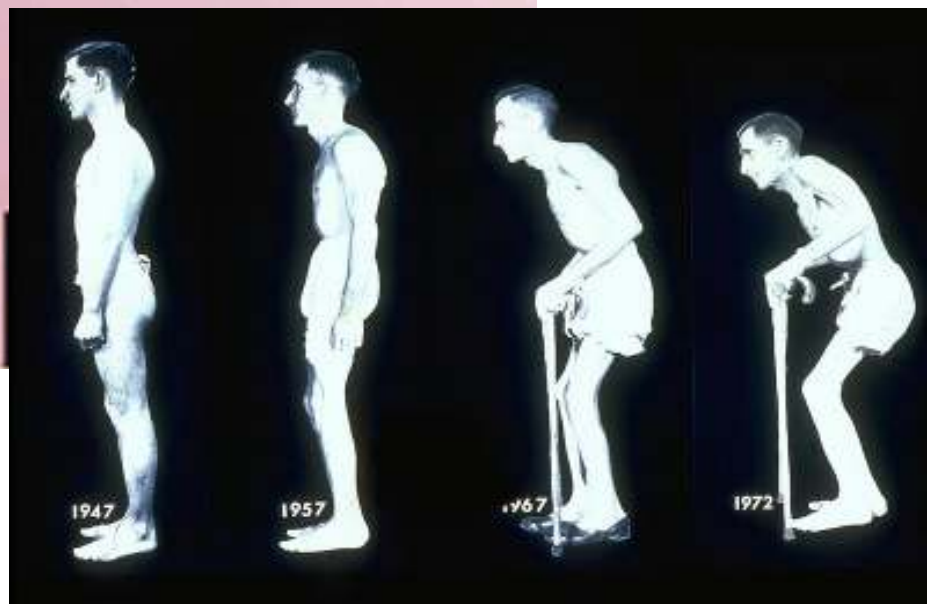


Inhibitors of co-stimulation
Anti-CD40L, CTLA-4Ig, Blys,
Anti-LFA1

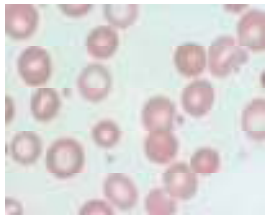
Dramatic inhibition of inflammation, bone damage, decrease in pain and improved function.
Remission if used early in 50% of patients

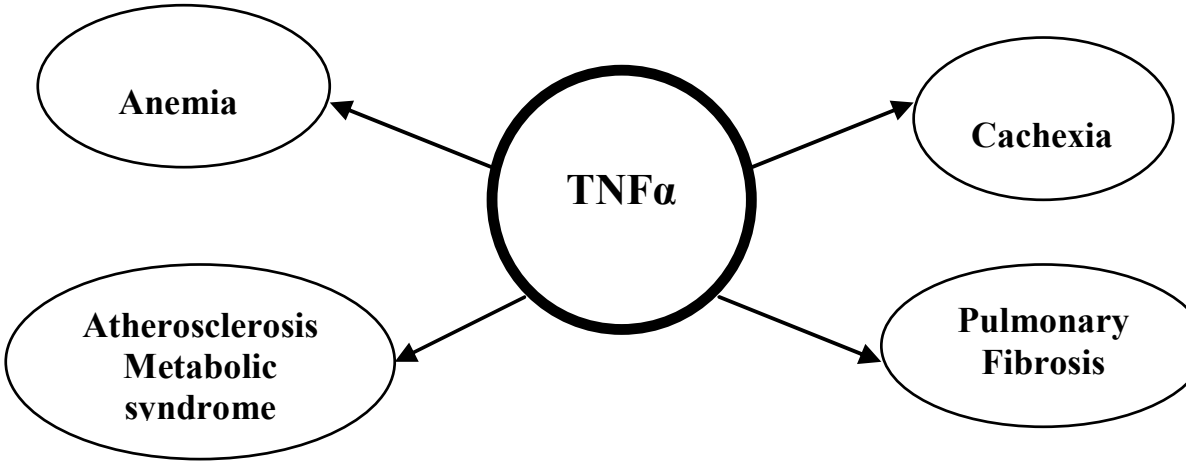


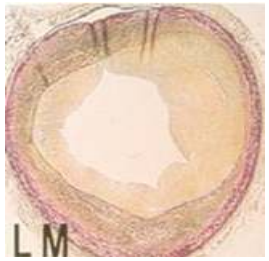


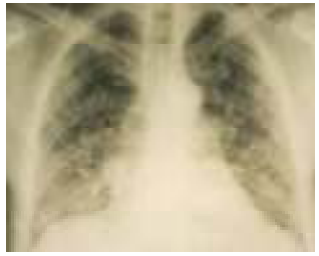


TNF is an important mediator in humans
TNF mediates many of the systemic effects of RA









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- **Inflammatory and autoimmune diseases are complex: the use of high-throughput methods in their investigation**
- **Perspective**

Immune responses are complex!!!!!!!

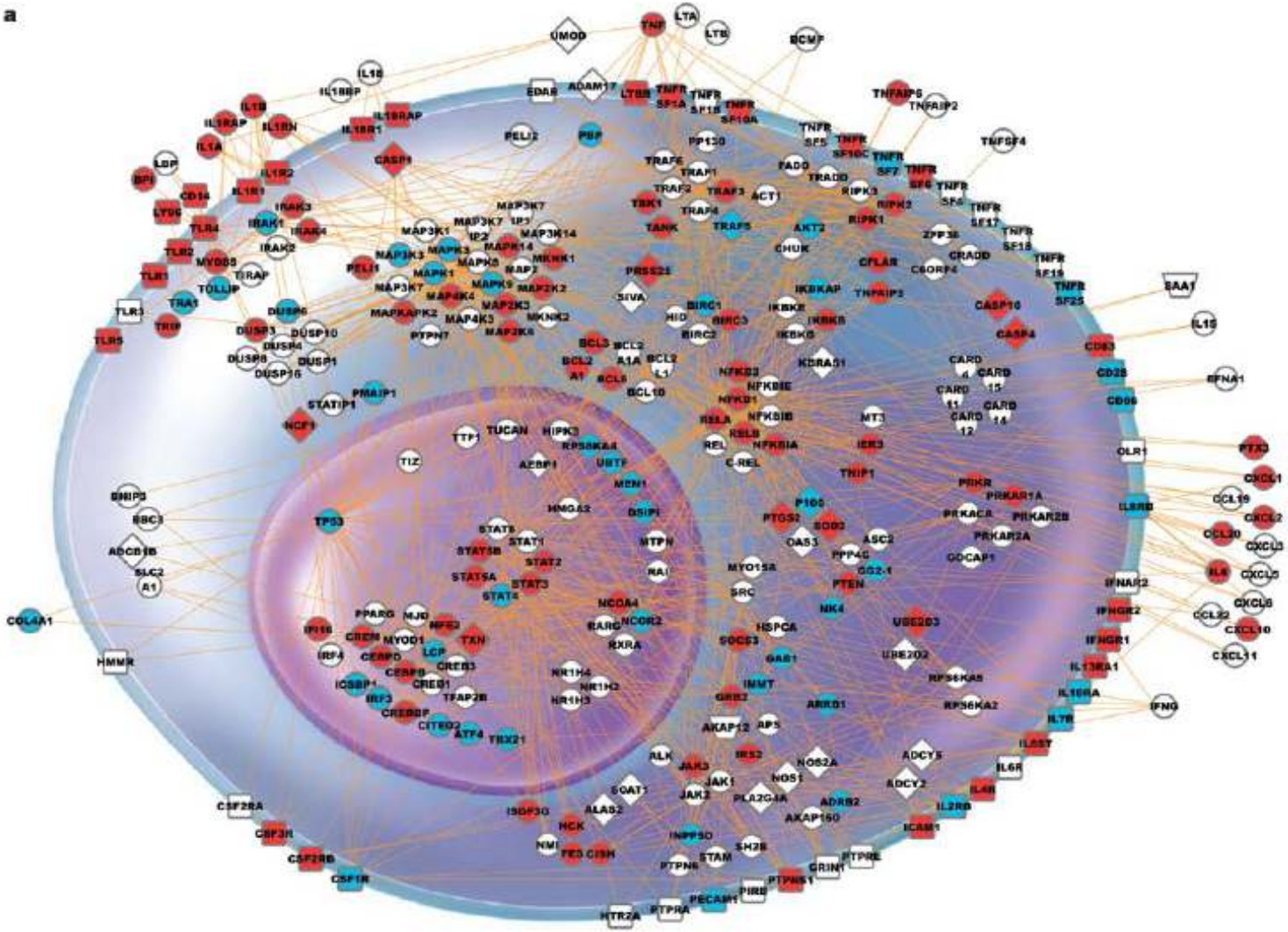
nature Vol 437|13 October 2005|doi:10.1038/nature03985

LETTERS

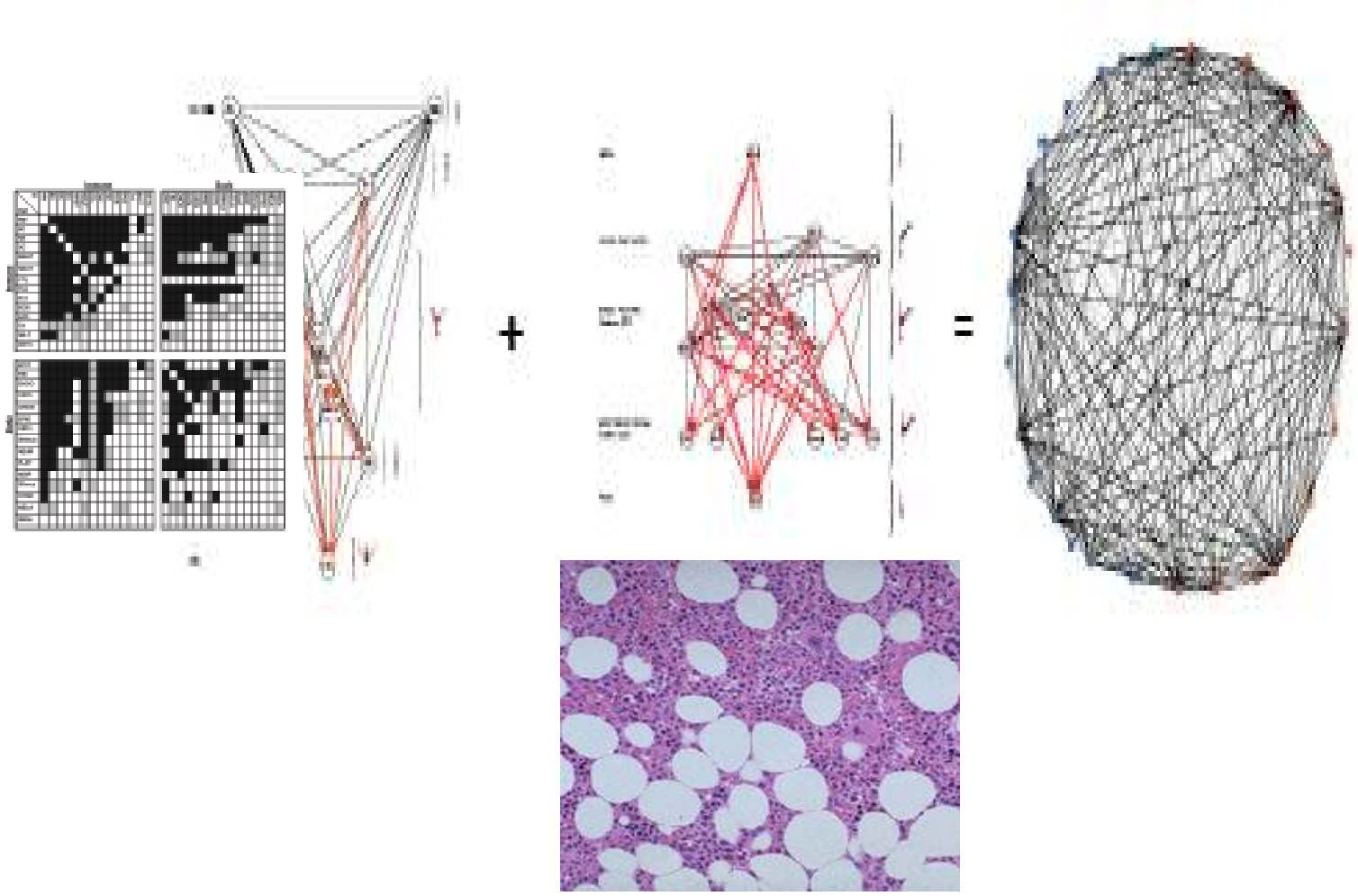
A network-based analysis of systemic inflammation in humans

Four healthy persons , iv endotoxin and analysis of human leukocytes

Prototypical inflammatory cell 292 genes, red up, blue down-regulation



The complexity of the system



Cells and molecules interact with each other and with ECM. Complexity resembles that of the CNS

Autoimmune rheumatic diseases are even more complex!!!!

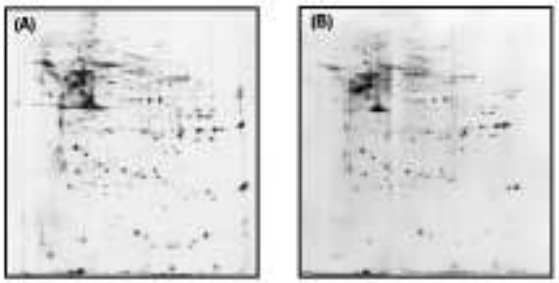
- **Rheumatic diseases are of complex aetiology with environmental and genetic factors interacting with each other**
- **Patients vary with regard to disease manifestations, age of onset, prognosis and therapeutic response**
- **Disease phenotype is a consequence of 100s-1000s gene expression changes in multiple affected tissues and immune effector cells**

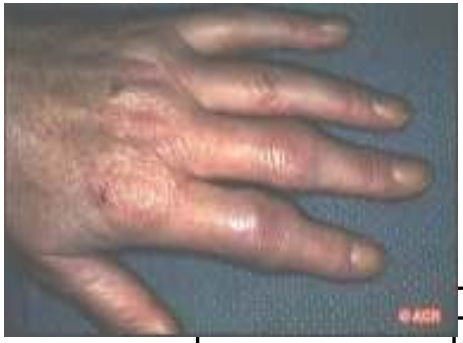
High throughput technologies are required

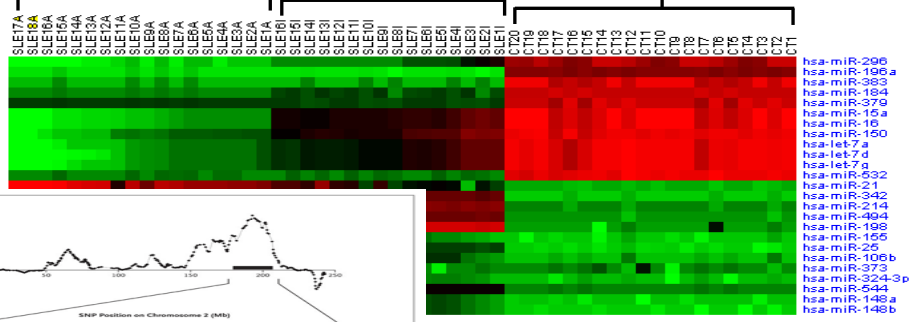
- **Thus far rheumatic disease research has been mainly focused in the investigation of specific molecules and inflammatory pathways**
- **Understanding the complex nature of rheumatic diseases as well as the implication of both genetic and environmental factors requires high throughput technologies**
- **High throughput technologies represent combinations of basic biological methods with automated biochemical, biological, optical and imaging methods**

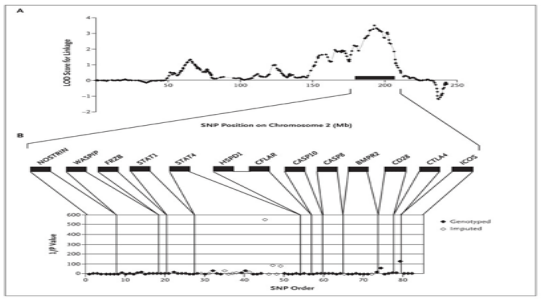
High throughput technologies in Rheumatology

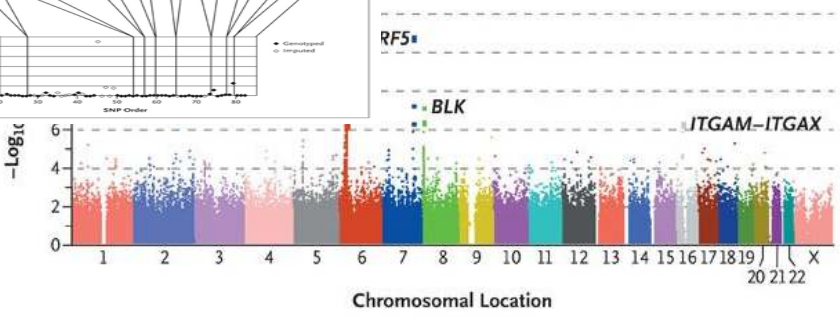
Rheumatoid arthritis
Proteomics and DNA microarrays



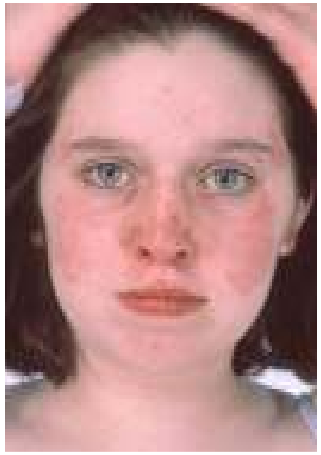


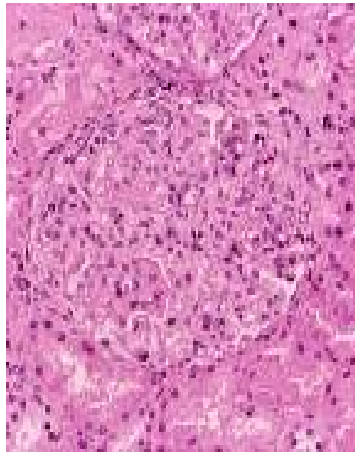






Systemic lupus erythematosus
miRNA and genome-scans



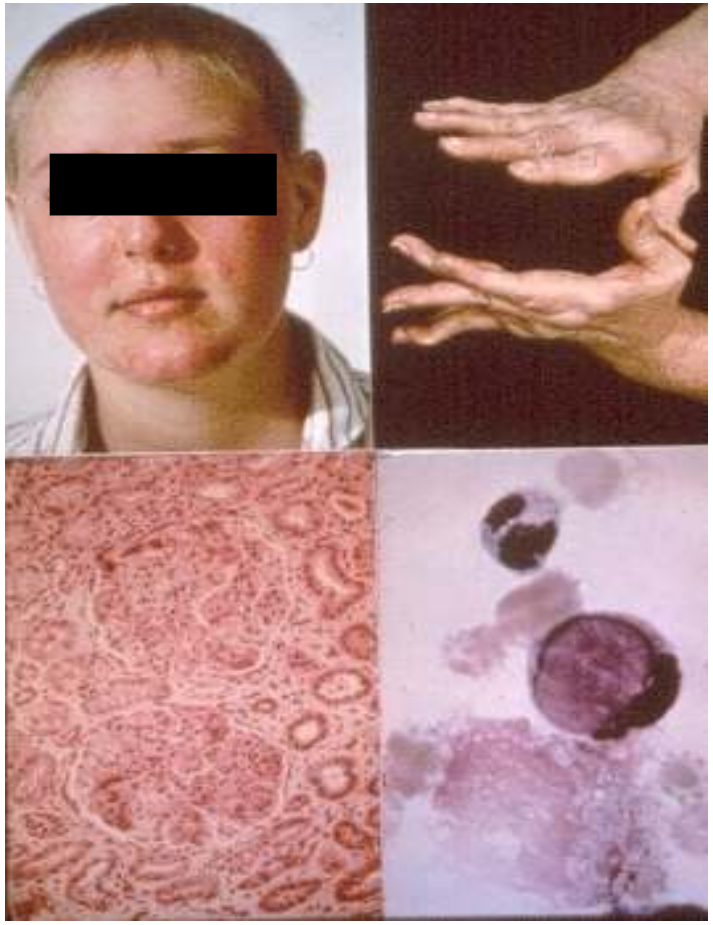


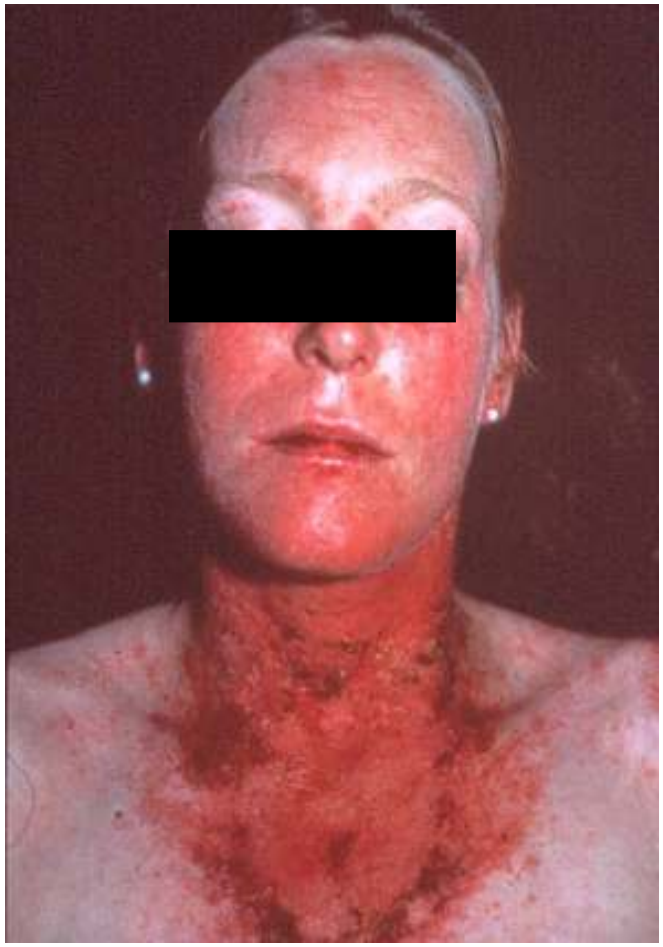
Outline

Questions to be addressed

- High-throughput technologies
  - Why are they necessary?
    - When to use? Circumstances and types of questions or problems
      - Which one ? Selection of method and technology
        - How to make sense of the results? Interpretation and integration

Lupus is the prototypic systemic autoimmune disease affecting a affecting multiple organs

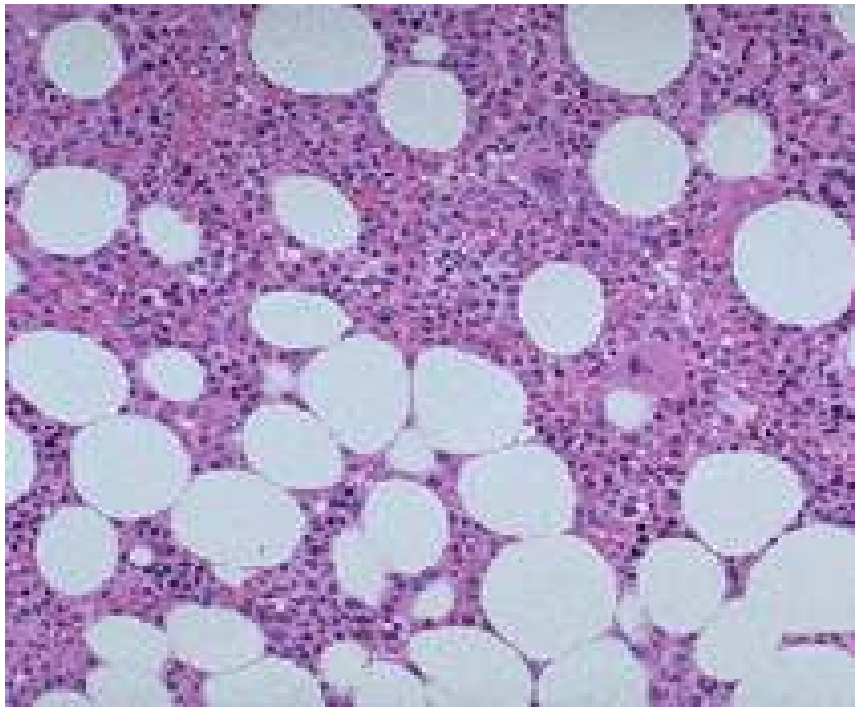




Bone Marrow: an ideal site to study the biology of lupus

- **Central lymphoid organ**:
  - » hemopoietic cells
  - – non hemopoietic cells
  - – the stroma

- **Important for the biology of B and T cells**



Approaches for identifying SLE candidate genes

Level 1: Polymorphisms

-***Genome-wide association studies***

- **Level 2: Gene expression**

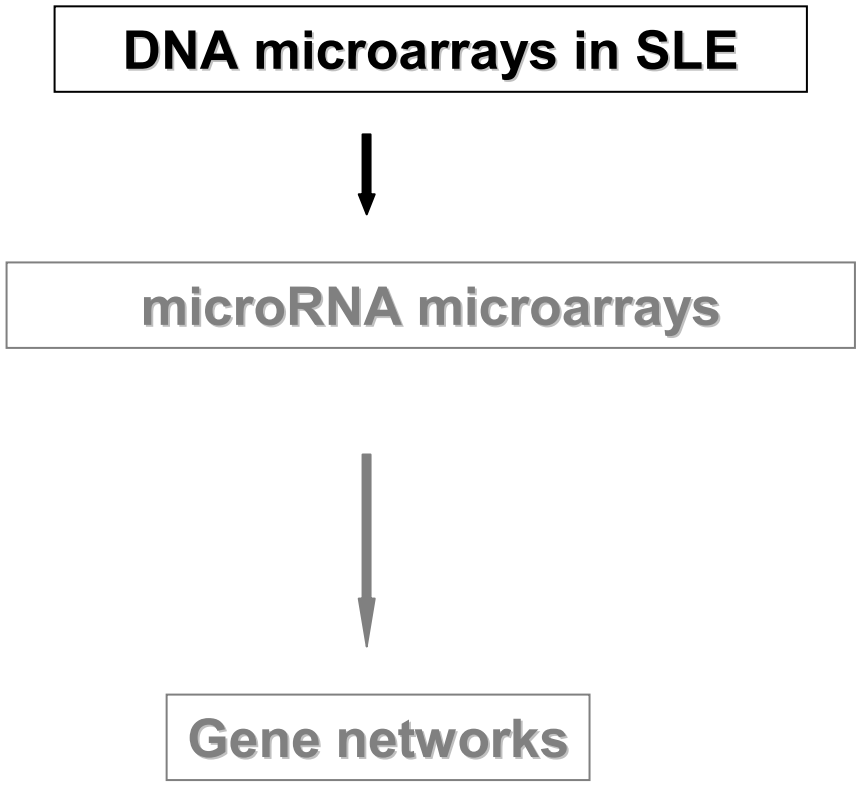
-***cDNA microarrays***

- **Level 3: Regulation of gene expression**

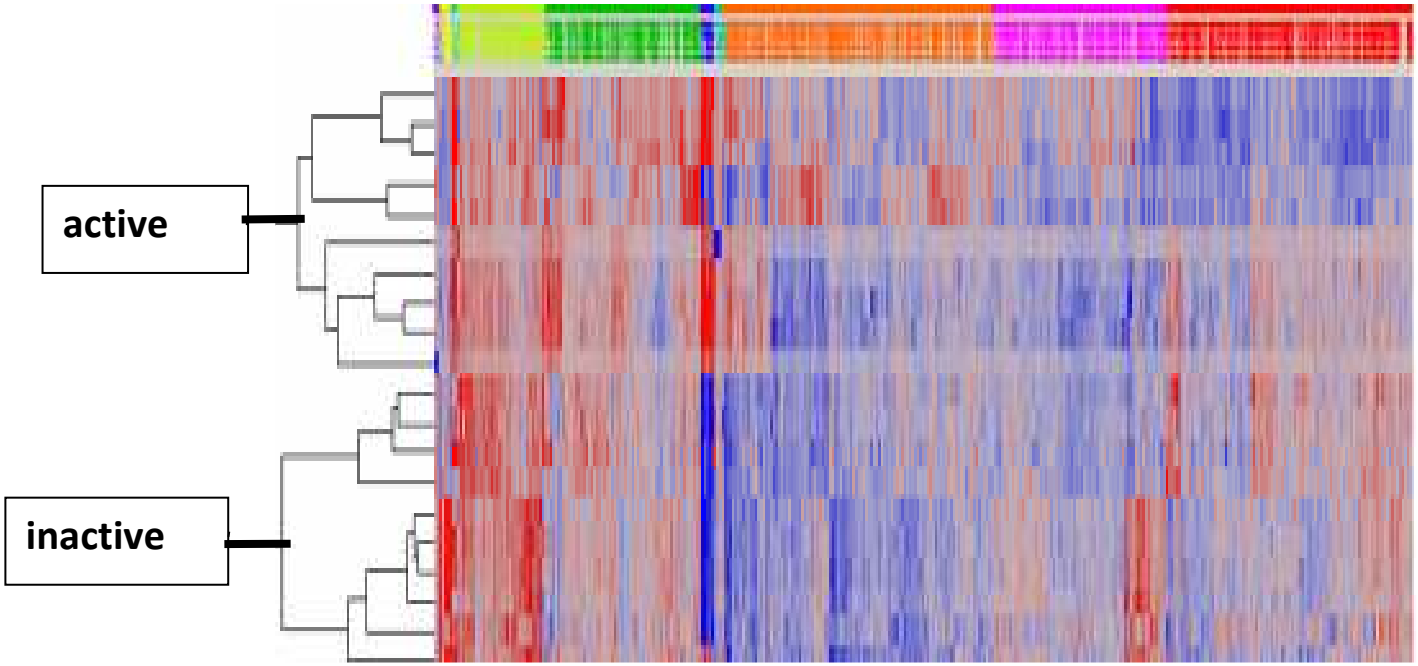
-***post-transcriptional, translational, post-translational***

- **Level 4: Proteomics**

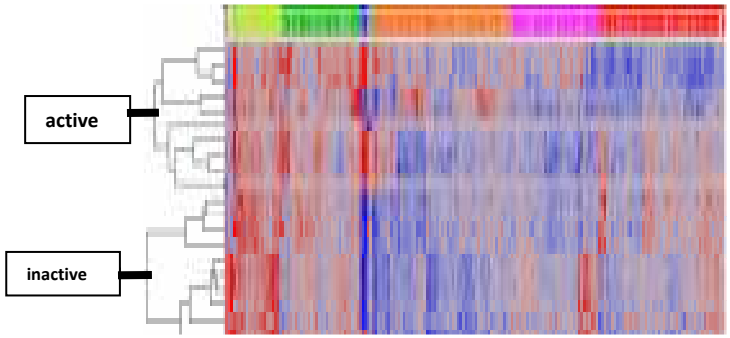
- serum and/or tissue


DNA microarrays in SLE
microRNA microarrays
Gene networks

Microarray analysis reveals patient subgroups in the Bone Marrow



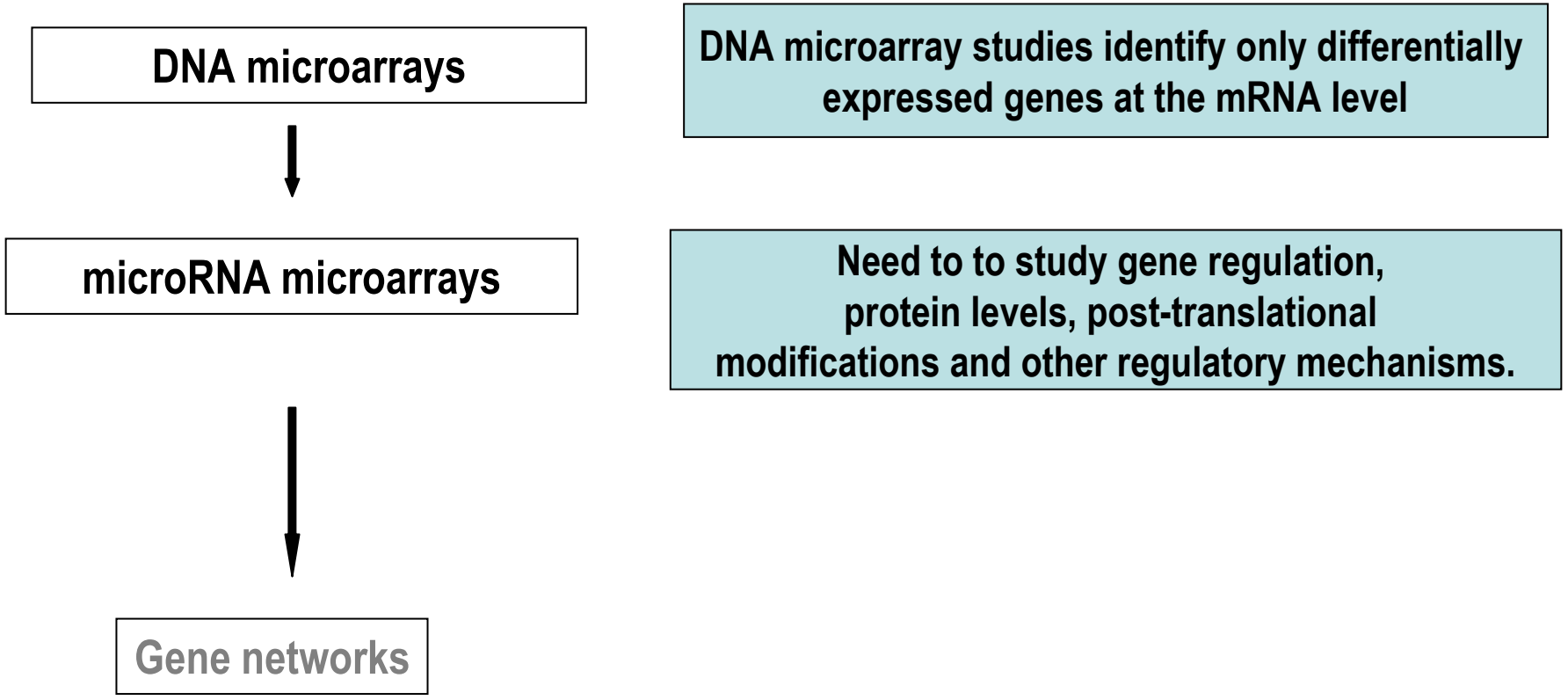
BM Genes differentially expressed according to disease activity

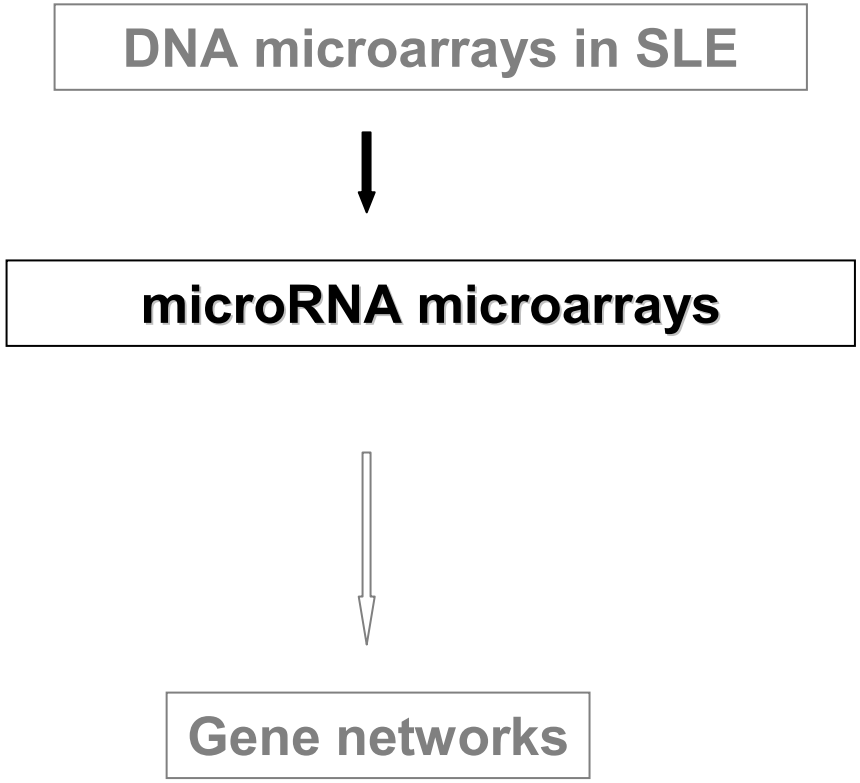


Granulopoiesis

Apoptosis of granulocytes

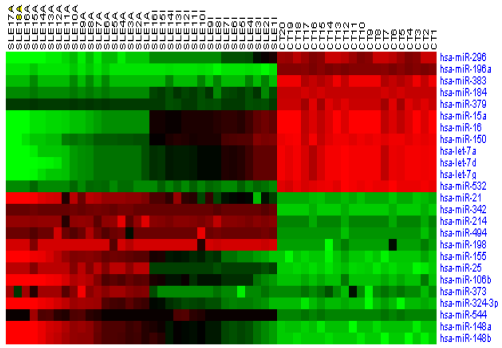
Integrin signaling


DNA microarrays
DNA microarray studies identify only differentially expressed genes at the mRNA level
microRNA microarrays
Need to to study gene regulation, protein levels, post-translational modifications and other regulatory mechanisms.
Gene networks

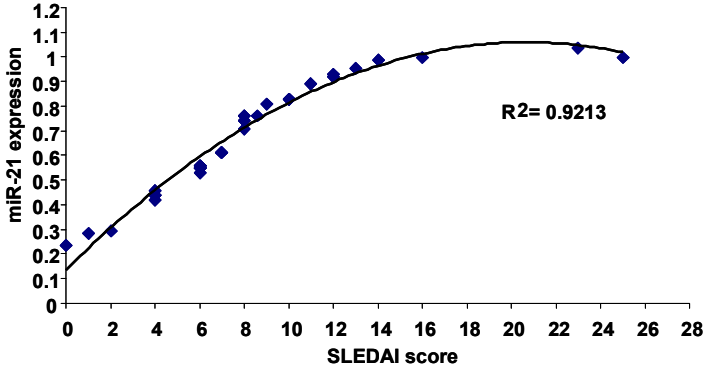

DNA microarrays in SLE
microRNA microarrays
Gene networks

microRNA microarrays

MicroRNA gene signature distinguishes SLE active vs inactive patients



Specific MicroRNAs correlate with SLE disease activity



Identification of gene targets involved in specific signaling pathways.

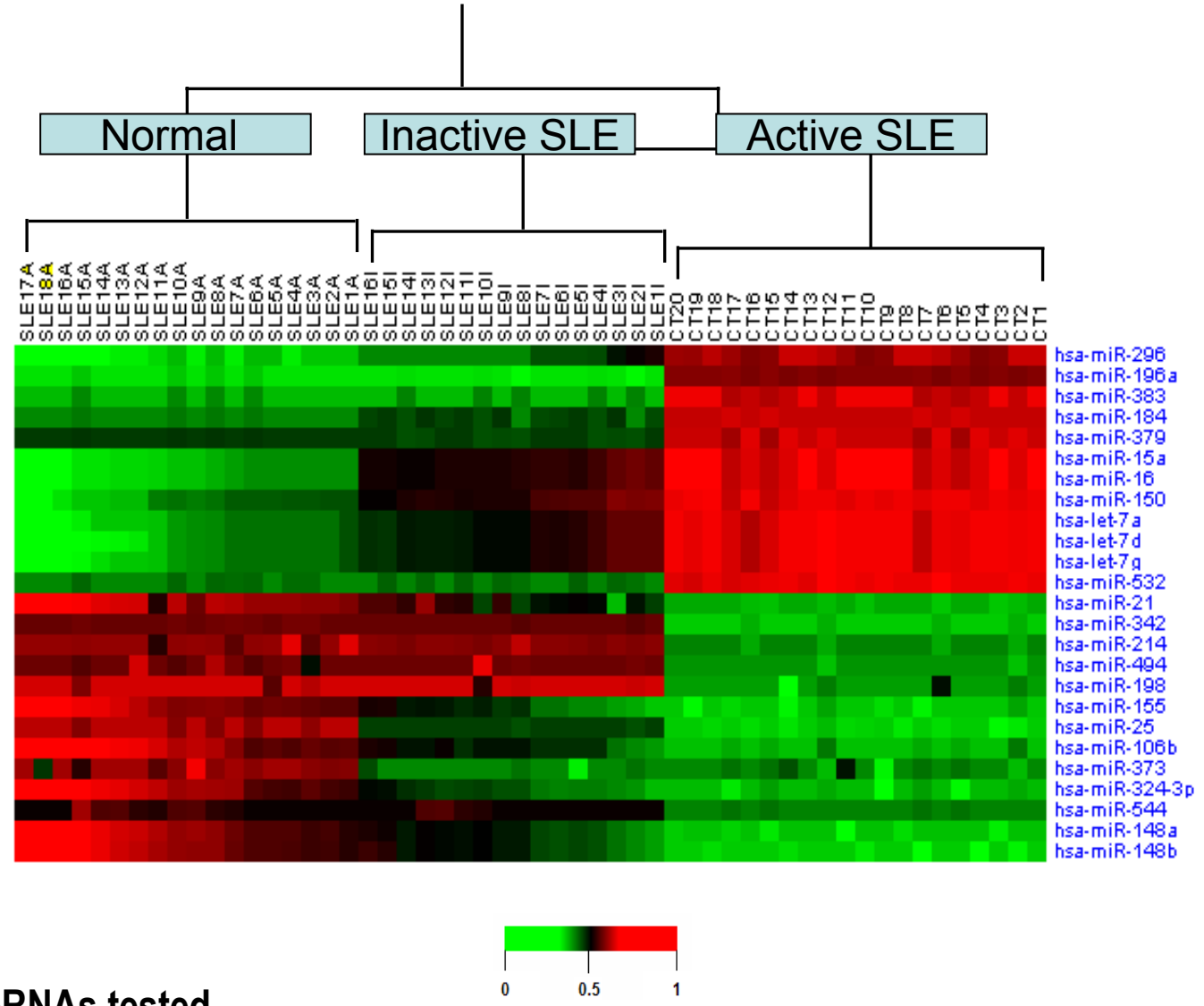
miR-16 → Bcl2, bcl-xl

miR-21 → PDCD4

miR-25 → BIM

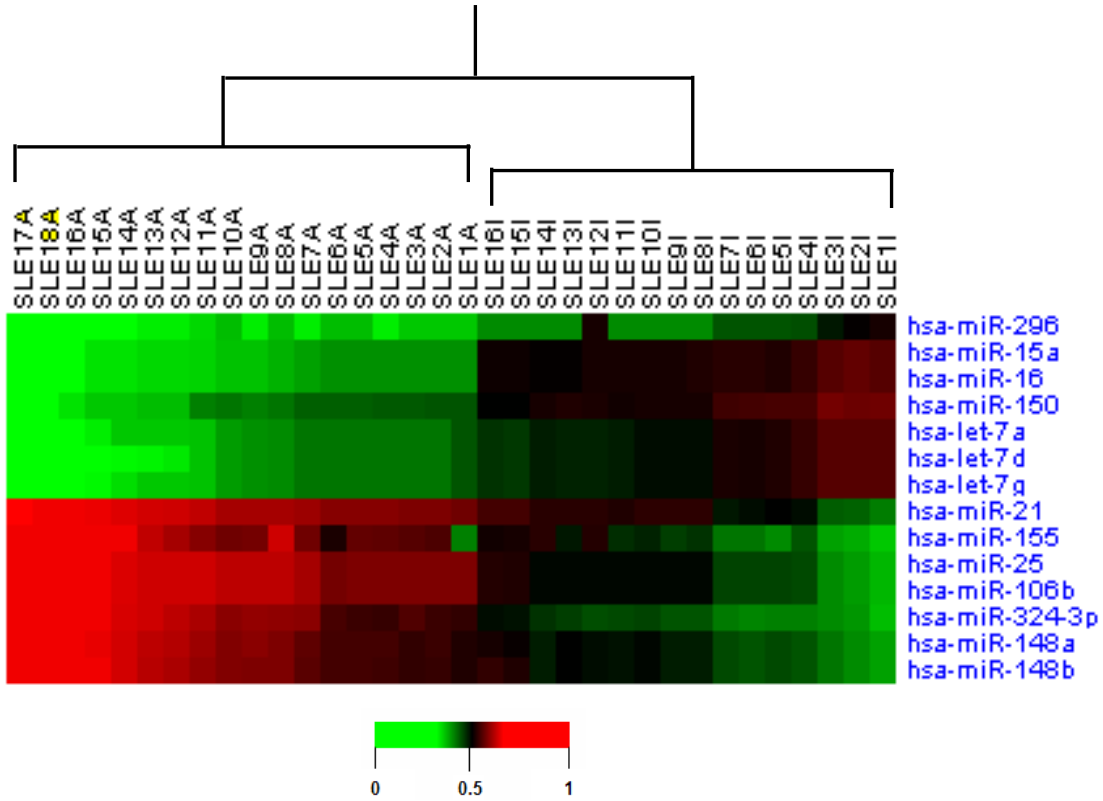
Implicated in apoptosis

25 MicroRNA Gene Signature Distinguishes Normal from SLE patients



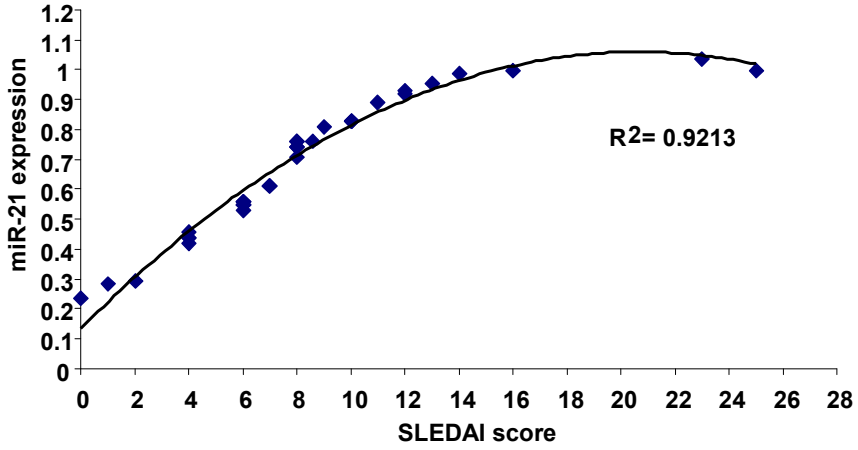
- 365 microRNAs tested
- 25 differentially expressed between normal and SLE patients
- 12 microRNAs were down-regulated and 13 were up-regulated in SLE patients

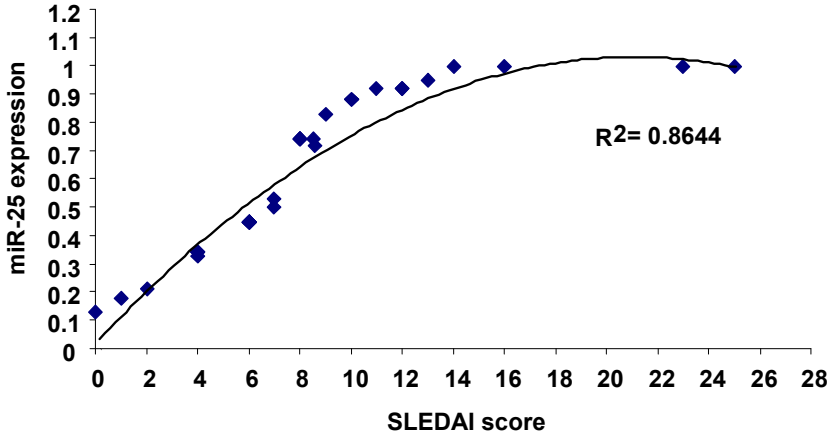
14 MicroRNA Gene Signature Distinguishes SLE active vs inactive patients

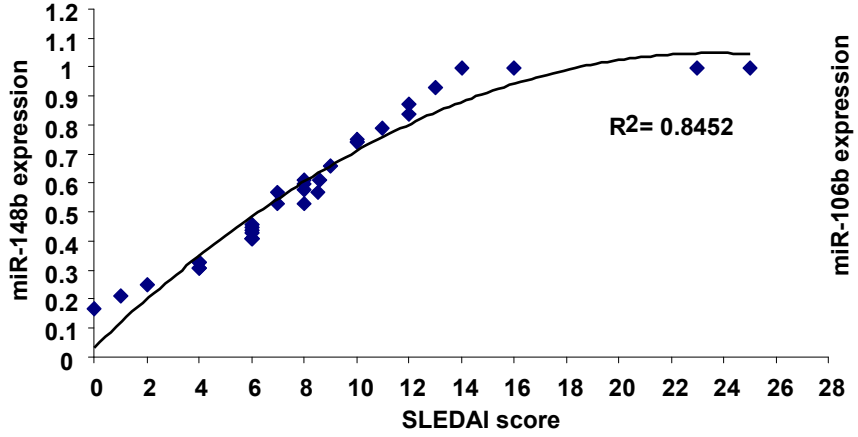


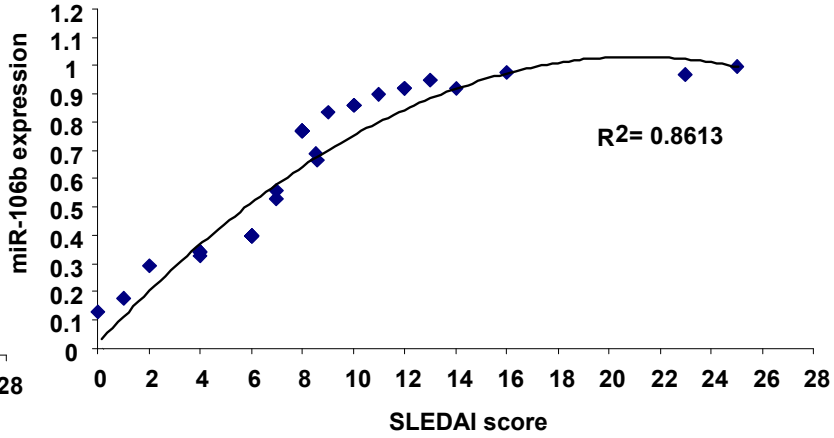
7 microRNAs were down-regulated and 7 were up-regulated in SLE patients with active disease in comparison to SLE patients with inactive disease (SLEDAI<8).

MicroRNAs correlate with SLE disease activity









4 microRNAs (miR-21, miR-25, miR-106b, miR-148b) are highly correlated with SLE disease activity. Probably these microRNAs can be used as SLE disease activity prognostic markers.

Identification of microRNA gene targets

MicroRNA gene	Chromosomal location[1]	Putative Targets[2]	Description
hsa-miR-150	19 : 54695854-54695937	c-myb	Myeloblastosis viral oncogene homolog
hsa-miR-25	7 : 99529119-99529202	Bim	Bcl2-like 11
hsa-miR-106b	7 : 99529552-99529633	Bim	Bcl2-like 11
		PTEN	Phosphatase and tensin homolog
hsa-miR-21	17 : 55273409-55273480	PTEN	Phosphatase and tensin homolog
hsa-let-7a	22 : 44887293-44887366	IL6	Interleukin 6
hsa-miR-196a	12 : 52671789-52671898	TCF7	Transcription factor 7, T-cell specific
hsa-miR-148b	12 : 53017267-53017365	DNMT3b	DNA Methyltransferase 3b

[1]MicroRNA chromosomal location (mouse genome) according to the miRBase database from Sanger Institute.

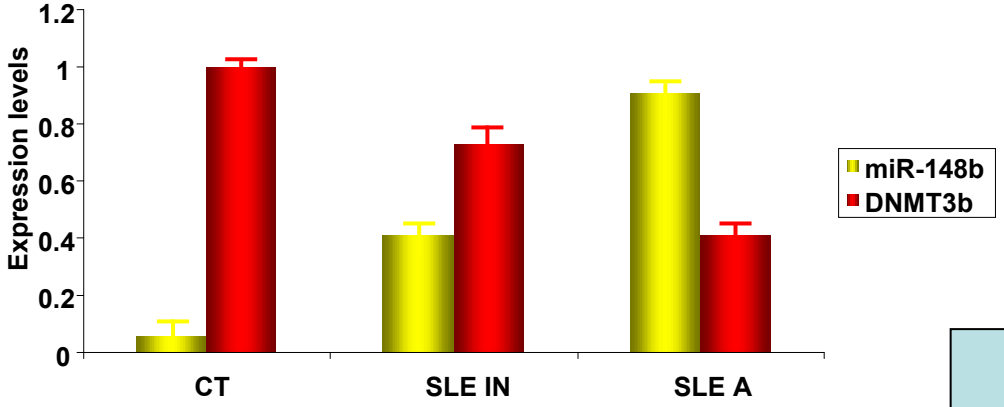
[2]Putative microRNA targets according to prediction algorithms and expression data.

To understand microRNAs function, need to identify gene targets involved in specific signaling pathways. By bioinformatic analysis we identified genes potentially targeted by the microRNAs differentially expressed in lupus

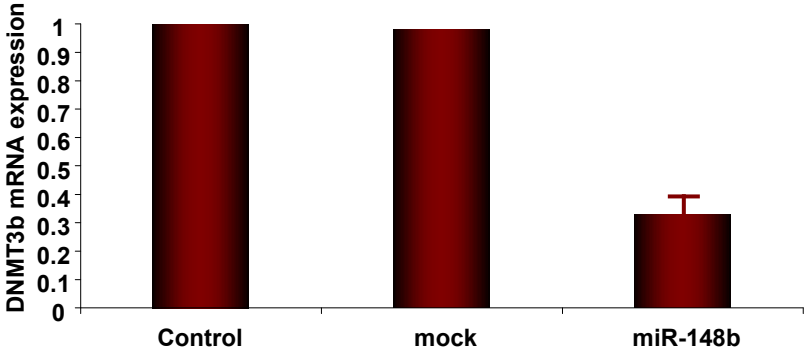
Validation of miRNA data

Overexpressionof miR-148b and testing DNMT3b expression levels

A



B

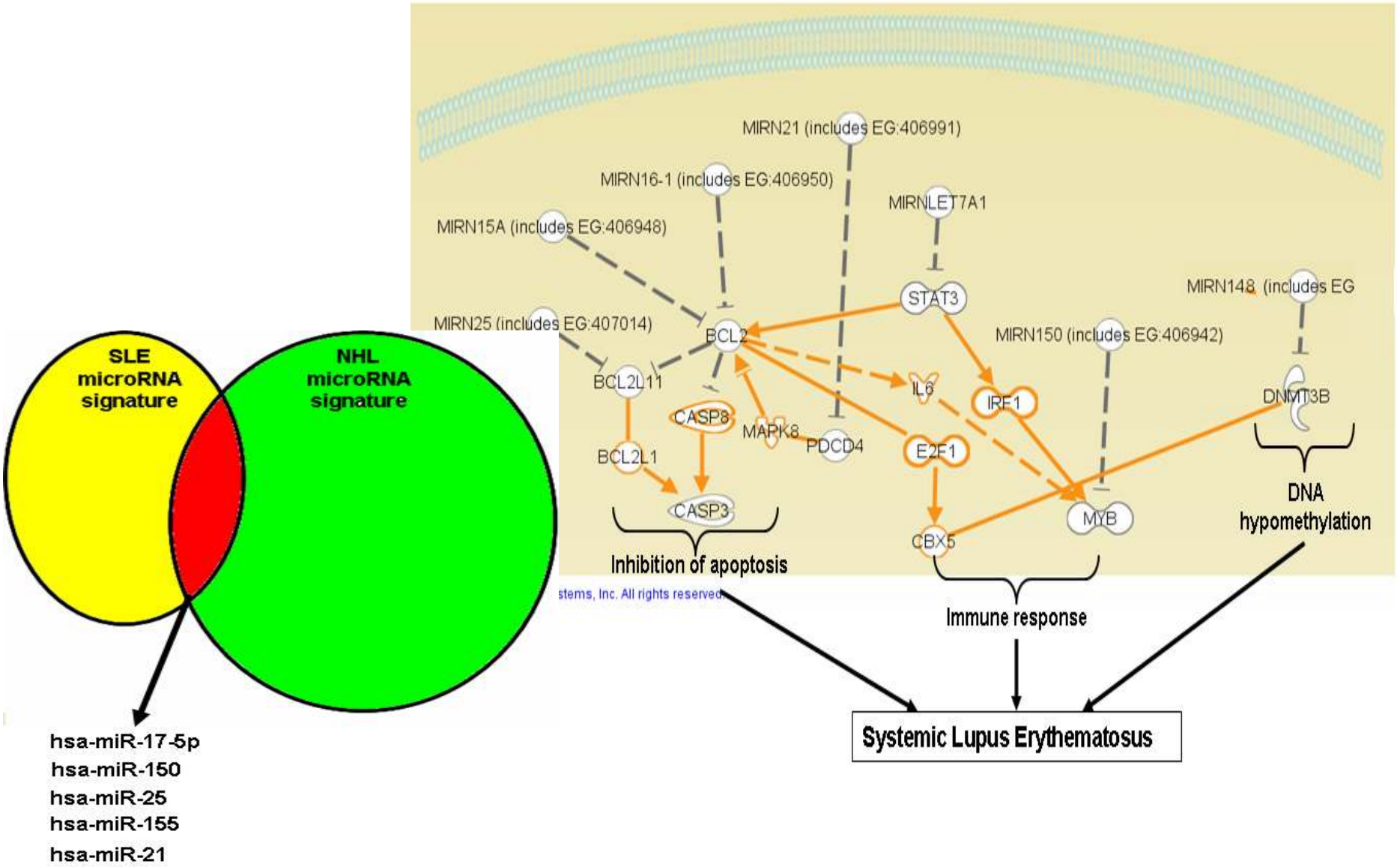


MicroRNA-induced hypomethylation in CD4+ T cells in lupus patients

miR-148b inhibited >70% DNMT3b mRNA expression assessed by real-time PCR analysis.

These results suggest that miR-148b targets directly DNMT3b and its up-regulation in SLE blocks DNMT3b ability causing global hypomethylation in CD4+ T cells.

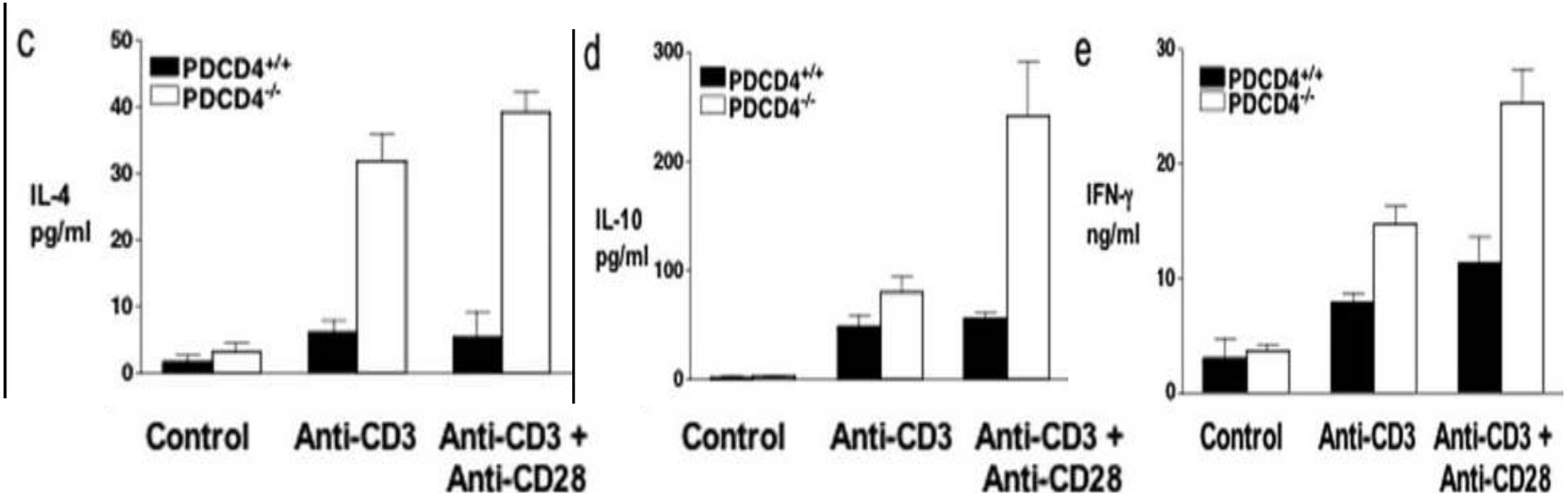
Lupus bionetwoorks



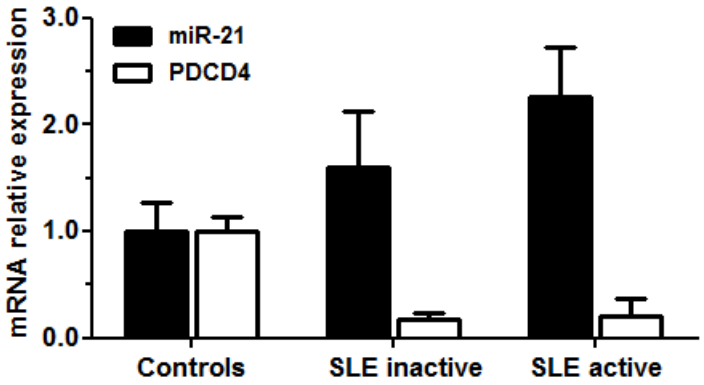
PDCD4 in the immune system

- Translational regulation of autoimmune inflammation and lymphoma genesis by PDCD4. *J Immunol*, 2006
  - *Spontaneous lymphoma development in PDCD4 ko mice*

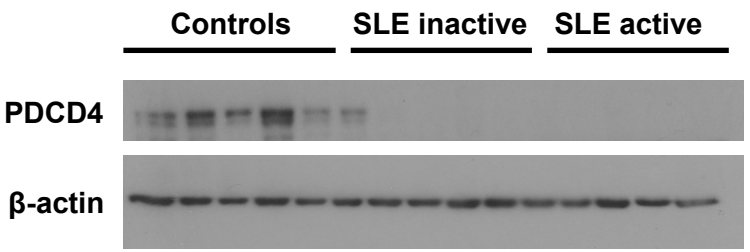
  - *PDCD4 deficient lymphocytes preferentially produce cytokines*



Mir-21 is inversed correlated with PDCD4 in PBMCs of SLE patients

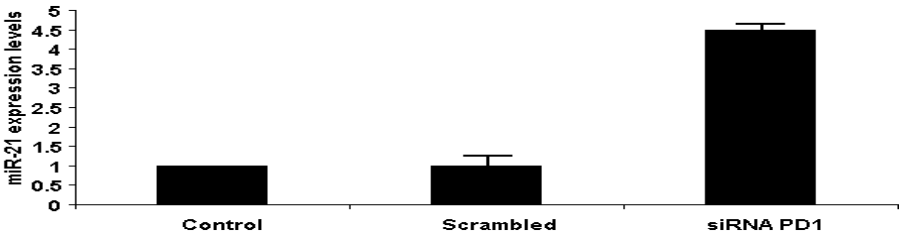


real time PCR analysis



Western blot analysis

PD1 inhibition increases miR-21 expression



Proposed model for PDCD1 (PD1) regulating miR-21

Normal **SLE**

mir-21 → PDCD4 → AP-1 → CD40L → hyperactivity of SLE T cells

Integrative Genomic Network Analysis Reveals Novel Drug Targets for SLE

- Gene profiling studies provide important information for detecting key molecules relevant to a disease BUT

  -they are not informative of protein-protein interactions, post-translational modifications and regulation by targeted sub-cellular localization.

  -in many diseases important proteins such as MAP kinases are activated by phosphorylation while their mRNA and proteins levels remain constant.

- We integrated gene expression profiling data, derived from bone marrow of lupus patients and healthy individuals, with bioinformatic approaches and constructed functional gene networks .

- Identification of the central nodes (also called hubs) in these networks could lead to the development of new drug therapies for lupus patients.

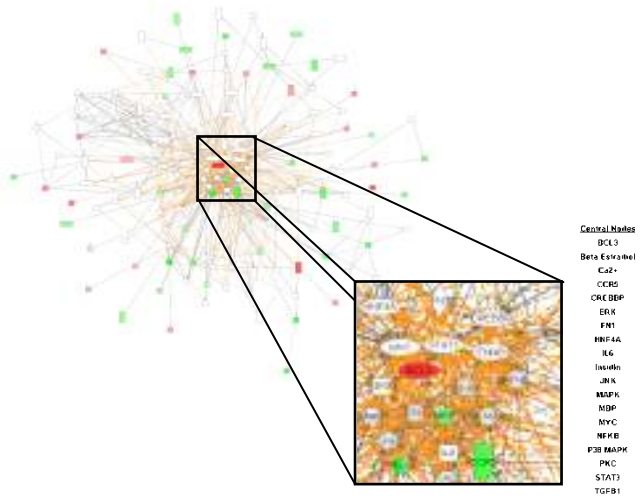
Patients and Methods

- Analysis of gene expression microarray data from bone marrow mononuclear cells (BMMCs) from 20 SLE patients (11 with active and 9 with inactive disease) and 7 healthy individuals and 3 osteoarthritis patients served as controls.

- Gene networks were constructed and identified important hubs using Ingenuity Gene Network Analysis.

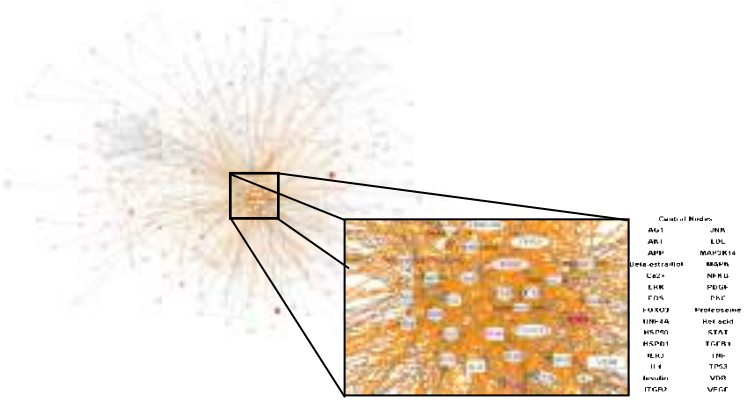
- Pathways of highly interconnected genes were identified by statistical likelihood

Gene networks



Control vs SLE patients

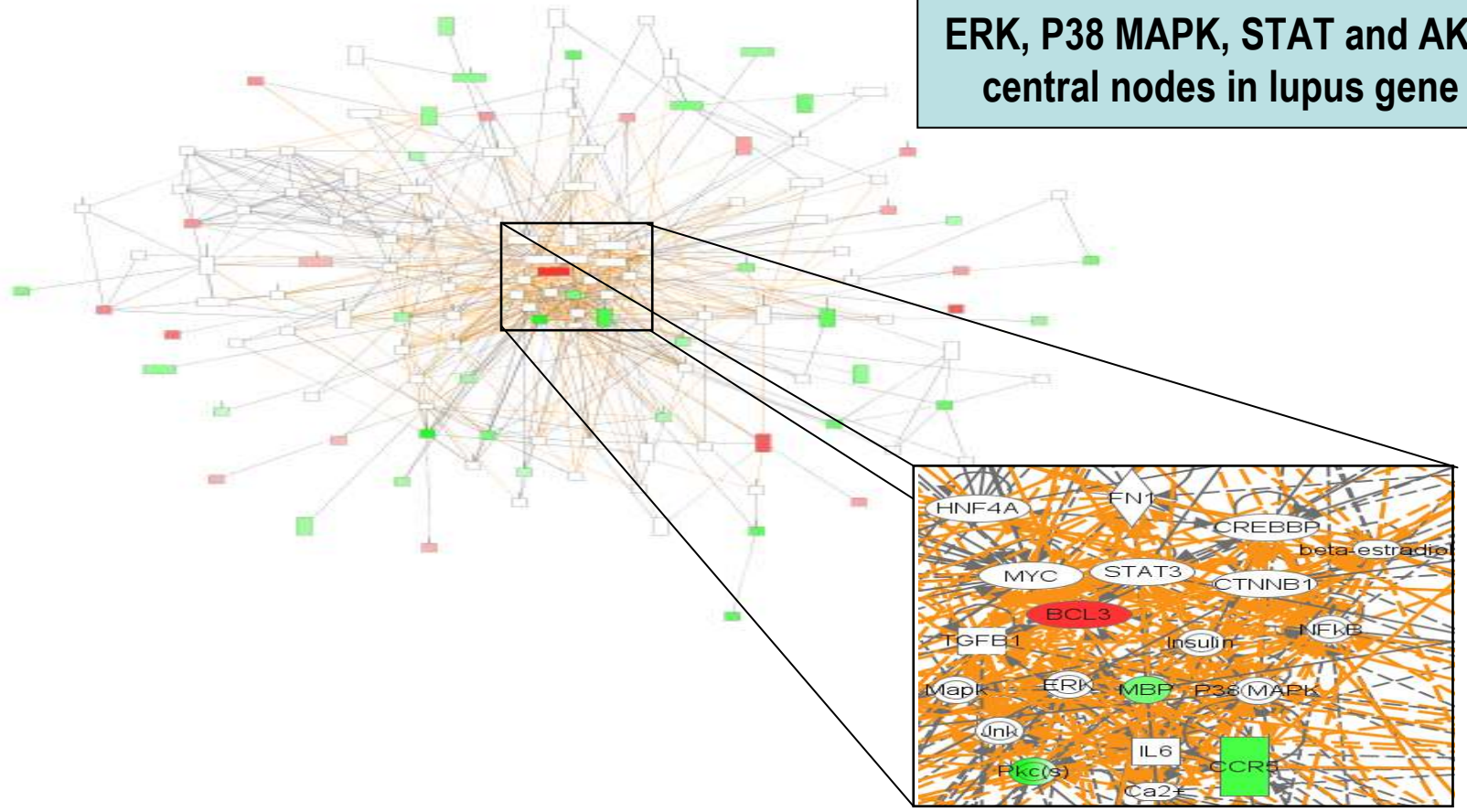
ID	*Score*	*Focus Molecules*	*Top Function*
1	35	16	Cell Growth
2	29	14	Nucleic Acid Metabolism
3	24	12	Amino Acid Metabolism
4	10	6	Protein Synthesis



SLE active vs inactive patients

ID	Score	Focus Molecules	Top Function
1	50	26	Energy Production
2	47	25	Carcinogenesis
3	40	22	Cellular Assembly and Organization
4	35	20	Immune Response
5	30	18	Cellular Growth and Proliferation
6	28	17	Protein Synthesis
7	26	16	RNA Post-Transcriptional Modification
8	18	12	Amino Acid Metabolism
9	14	10	Lipid Metabolism

ERK, P38 MAPK, STAT and AKT proteins, central nodes in lupus gene networks



Central Nodes

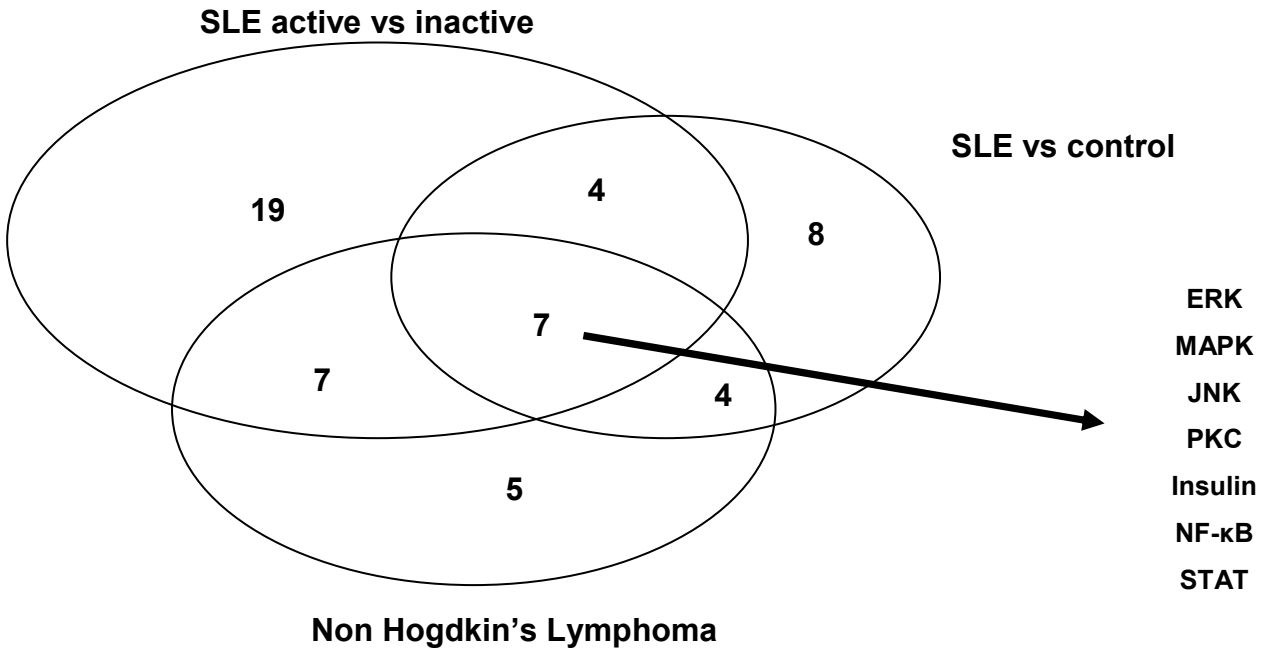
BCL3
Beta-Estradiol
Ca2+
CCR5
CREBBP
ERK
FN1
HNF4A
IL6
Insulin
JNK
MAPK
MBP
MYC
NFKB
P38 MAPK
PKC
STAT3
TGFB1

Gene Network Analysis Reveals Activation of Multiple Kinase Pathways

We tested the protein expression levels and activation of ERK, P38 MAPK, STAT and AKT proteins, which were central nodes in lupus gene networks, using protein extracted from B cells of control and NZB/W F1 female mice (4 months)SLE mouse model.

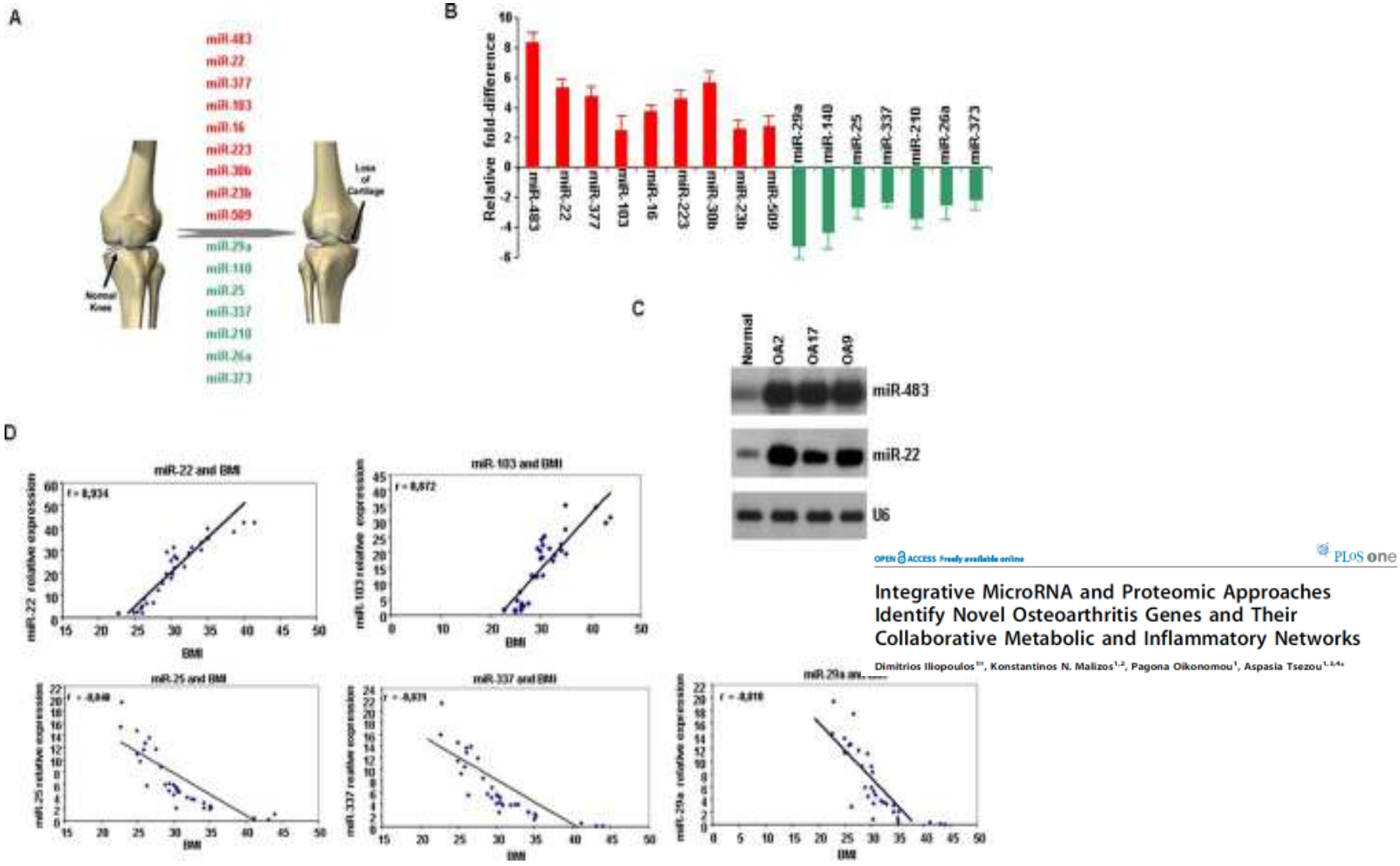
Validation of network data in lupus mice NZB/NZW by Western blotting

Using a literature-curated gene data set for Non Hodgkin's lymphoma we compared the similarity between lupus and Non Hodgkin's lymphoma gene networks and identified common central nodes.



Normal vs SLE
Active SLE vs inactive
SLE vs Non-Hodgkin lymphoma

miRNA in human osteoarthritis and correlation with BMI



OPEN ACCESS Freely available online

PLoS one

Integrative MicroRNA and Proteomic Approaches Identify Novel Osteoarthritis Genes and Their Collaborative Metabolic and Inflammatory Networks

Dimitrios Iliopoulos[1*], Konstantinos N. Malizos[1,2], Pagona Oikonomou[1], Aspasia Tsezou[1,3,4*]

Obesity and inflammation are related to osteoarthritis, a metabolic disease affected by microRNA deregulation.

High-throughput technologies : time for clinical application?

- **Time, expense and expertise required to do the assays are considerable obstacles**
- **Standardization and validation problematic especially in view of the scarcity of the scarcity of current bioinformatics expertise**
- **Clinical application: Further validation whether**

-a single assay on an individual patient will be even be interpretable (most data are derived from comparing groups opf patients in a cross-sectional rather than longitudinal fashion)

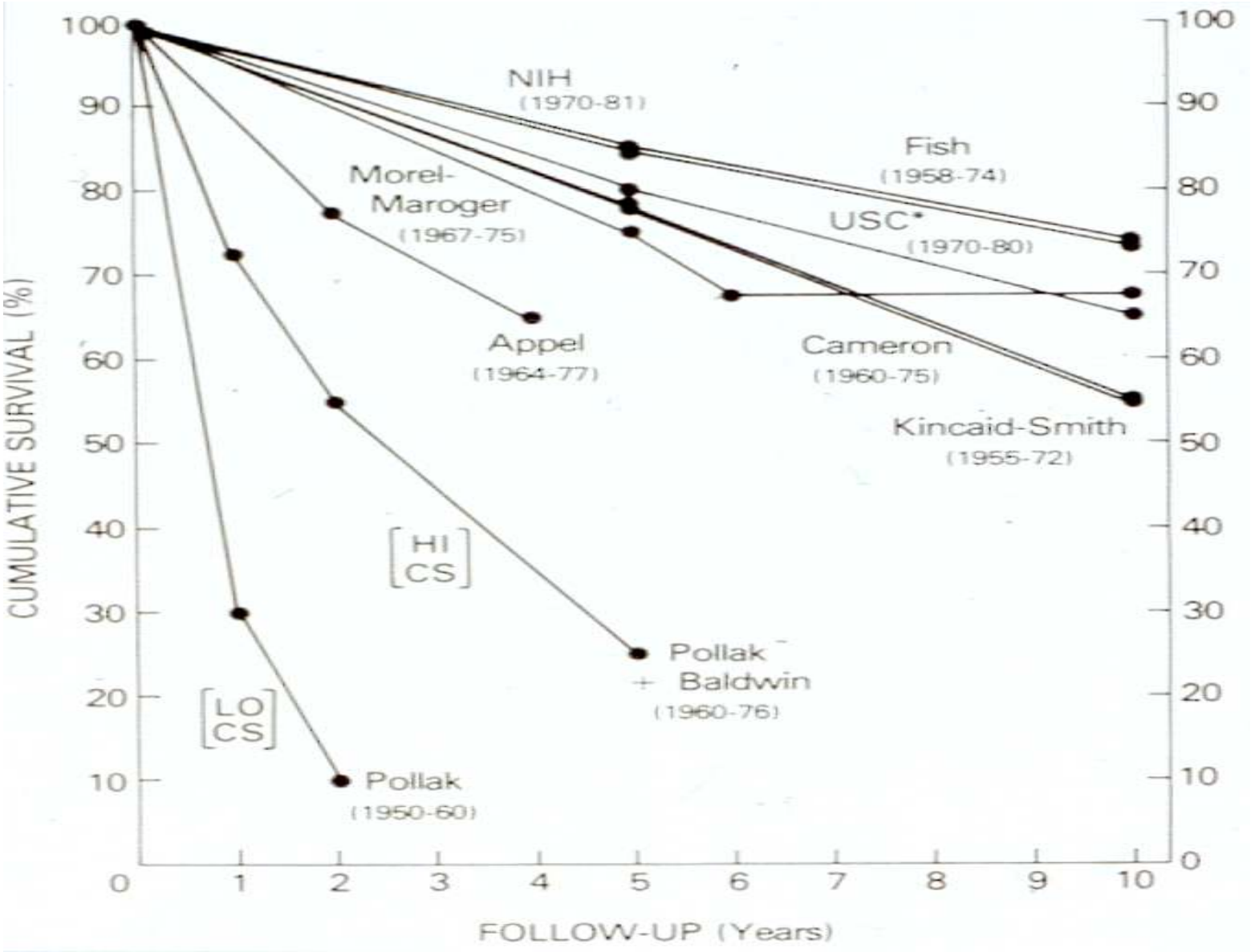
-its discriminant ability towards similar diseases or towards disease activity

Immunity, Inflammation and Autoimmunity in Humans

Physiology, pathophysiology, nosology and therapeutics

- **Origin and physiologic role of inflammation**
- ***Exogenous* inflammation vs *endogenous* inflammation and associated diseases**
- ***Endogenous inflammation*: Auto-inflammation vs autoimmune inflammation**
  - **Auto-inflammatory diseases: Diseases of innate immunity**
  - **Autoimmune diseases: Diseases of innate and adaptive immunity**
- **Biologic therapies: Lessons learned about the targeting of key molecules and cells**
- **Inflammatory and autoimmune diseases are complex: the use of high-throughput methods in their investigation**
- **Perspective**

10 Year Survival in SLE



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- **Wyeth**

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