Autoimmunity and Inflammation

From bench to bed-side

Dimitrios T Boumpas, MD, FACP
University of Crete; University Hospital of Heraklion; IMBB, FORTH; and Graduate Program “Molecular Basis of Human Diseases
Immunity and its homeostatic regulation

- The immune system is among the most fundamental requirements for survival. Thus, it is 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)
  
  - Host-derived stress (stress associated molecular patterns, SAMPs)

Host-derived stress signals: by-products of apoptotic cells (hsp, histone 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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- Perspective
Inflammation underlies a wide variety of physiological and pathological processes.

Although the pathological aspects of many types of inflammation are well appreciated, their physiological functions are mostly unknown.
Cellular states and surveillance of tissues by ΜΦ

Blue arrows indicate signals that report the tissue state to ΜΦ; Red arrows indicate ΜΦ-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, adipocytes 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

- **Para-inflammation**
  - Increase in MΦ- plasma proteins
  - Endothelial dysfunction

- **Inflammation**
  - Infiltration by inflammatory cells
  - Monocytes, lymphocytes

- **Tissue surveillance by macrophages**

- **Infection**
  - Tissue injury
  - Auto-immunity
  - Auto-inflammation

**Images:**
- Normal artery
- Mild hypertension
- Severe hypertension
- Tissue section
- Histological section
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.
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.
• **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

Autoimmune

- self-reactivity
  - (autoreactive B and T cells, autoantibodies)

- SLE, RA

Autoinflammatory

- No self-reactivity

- FMF

Adaptive immunity

Innate

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.

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 NALP3 inflammasome is a crucial element in the adjuvant effect of aluminum and can direct a humoral adaptive immune response.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene (chromosome)</th>
<th>Protein (synonyms) or pathogenic stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 1: IL-1β activation disorders (inflammasomopathies)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intrinsic</strong></td>
<td></td>
<td></td>
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<tr>
<td>FCAS&lt;sup&gt;5&lt;/sup&gt;, MWS&lt;sup&gt;3&lt;/sup&gt;, NOMID&lt;sup&gt;6&lt;/sup&gt;/CINCA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>NLRP3/CASLI (1q44)</td>
<td>NLRP3&lt;sup&gt;5&lt;/sup&gt; (cryopyrin, NALP3, PYPAF1)</td>
</tr>
<tr>
<td>FMF&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAPA&lt;sup&gt;2&lt;/sup&gt;</td>
<td>PSTPIP1 (15q24–25.1)</td>
<td>PSTPIP1&lt;sup&gt;2&lt;/sup&gt; (CD2BP1&lt;sup&gt;2&lt;/sup&gt;)</td>
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<tr>
<td>CRMO&lt;sup&gt;1&lt;/sup&gt;/SAPHO&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Complex</td>
<td></td>
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<tr>
<td>Majeed syndrome</td>
<td>LIPIN2 (18p11.31)</td>
<td>Lipin-2</td>
</tr>
<tr>
<td>HIDS&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MVK (12q24)</td>
<td>Mevalonate kinase</td>
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<td>Recurrent hyalidiform mole</td>
<td>NLRP7 (19q13)</td>
<td>NLRP7 (NALP7, PYPAF3, NOD12)</td>
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<tr>
<td>DRA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>IL1RN</td>
<td>IL-1Ra</td>
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<td><strong>Complex/acquired</strong></td>
<td>Complex</td>
<td></td>
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<tr>
<td>Gou, pseudogou</td>
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<td>Uric acid/CPPO</td>
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<tr>
<td>Fibrosing disorders</td>
<td>Complex</td>
<td>Adenosis/ silica</td>
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<tr>
<td>Type 2 diabetes mellitus</td>
<td>Complex</td>
<td>Hyperglycemia</td>
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<tr>
<td>Schnitzler syndrome</td>
<td>Sporadic</td>
<td></td>
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</tbody>
</table>

[Diagram of IL-1β activation disorders]
FMF: Clinical Features

1-3 day episodes of fever with:

• Abdominal pain
• Chest pain
• Arthritis
• Rash

Peritonitis (air-fluid levels)

Pleurisy (left pleural effusion)

Posterior pericardial effusion

Chronic arthritis of the hip

Erysipeloid erythema
FMF: mutation in the pyrin gene

a

b

Predicted structure of the pyrin B30.2/SPRY domain
| 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 |

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Sidiropoulos et al Ann Rheum Dis 2007
<table>
<thead>
<tr>
<th>Type 2: NF-κB activation disorders</th>
<th>Gene</th>
<th>Stimulus</th>
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<tbody>
<tr>
<td>Crohn's disease</td>
<td>Complex</td>
<td>Muramyl dipeptide</td>
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<tr>
<td></td>
<td>NOD2 (16p12)</td>
<td>NOD2&lt;sup&gt;2&lt;/sup&gt; (CARD15)</td>
</tr>
<tr>
<td></td>
<td>ATG16L1 (2q37.1)</td>
<td>ATG16L1&lt;sup&gt;p&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>IRGM (5q33.1)</td>
<td>IRGM&lt;sup&gt;p&lt;/sup&gt;</td>
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<tr>
<td>Blau syndrome</td>
<td>NOD2 (16p12)</td>
<td>NOD2 (CARD15)</td>
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<tr>
<td>FCAS2 (Guadeloupe periodic fever)</td>
<td>NLRP12 (19q13.4)</td>
<td>NLRP12 (NALP12)</td>
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</table>
Complement activation syndromes

<table>
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<tr>
<th>Type 4: Complement disorders</th>
<th>aHUS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Complement factor H</th>
<th>MCP (1q32)</th>
<th>MCP&lt;sup&gt;b&lt;/sup&gt; (CD46)</th>
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<tbody>
<tr>
<td></td>
<td>CFH (1q32)</td>
<td></td>
<td>CFI (4q23)</td>
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<td></td>
<td>CFB (6p21.3)</td>
<td></td>
<td>Complex</td>
<td>Autoantibodies</td>
</tr>
<tr>
<td></td>
<td>Complex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CFH (1q32)</td>
<td>Complement factor H</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> aHUS: Atypical Hemolytic Uremic Syndrome

<sup>b</sup> MCP: Masp2-Corin-Protase K System
Aberrant cytokine signaling: cherubism
Macrophage activating syndromes: Molecular lesions that affect their activation

<table>
<thead>
<tr>
<th>Type &amp; Macrophage activation</th>
<th>Gene (chromosome)</th>
<th>Protein (synonym) or pathogenetic stimulus</th>
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</thead>
<tbody>
<tr>
<td>Familial HLH²</td>
<td>UNC13D (17q21.1)</td>
<td>Munc13-4</td>
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<tr>
<td></td>
<td>PRF1 (10q21.1)</td>
<td>Perforin 1</td>
</tr>
<tr>
<td></td>
<td>STX11 (6q24.2)</td>
<td>Syntaxin 11</td>
</tr>
<tr>
<td></td>
<td>Complex</td>
<td>Virus</td>
</tr>
<tr>
<td></td>
<td>LYST (1q41.3)</td>
<td>LYST² (CHS1)</td>
</tr>
</tbody>
</table>

Defective cytotoxic T and NK cells

Scavenger macrophage

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene (chromosome)</th>
<th>Protein (synonym) or pathogenetic stimulus</th>
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</thead>
<tbody>
<tr>
<td>Griscelli syndrome</td>
<td>RAR27A (15q21.3)</td>
<td>RAR27A</td>
</tr>
<tr>
<td>X-linked lymphoproliferative syndrome</td>
<td>SH2D1A (Xq25)</td>
<td>SAP²</td>
</tr>
<tr>
<td>Hermansky-Pudlak syndrome</td>
<td>HPS1-3</td>
<td>HPS1-3²</td>
</tr>
<tr>
<td>Secondary HLH</td>
<td>Complex</td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Complex</td>
<td>Cholesterol</td>
</tr>
</tbody>
</table>
Autoimmunity

Inflammation

HEAT  REDNESS  SWELLING  PAIN  LOSS OF FUNCTION
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

Environment (30-70%)
Genetic background (30-70%)

Autoimmune disease

Other factors i.e. socioeconomic

Response to tissue injury and ability to repair

Phenotype Severity-Outcome
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

| Table 1 Genetic loci with confirmed associations with human autoimmune disorders |
|---------------------------------|-----------------|------------------|
| **Gene** | **Location** | **Function** | **Diseases** |
| Intracellular signaling and receptors | | | |
| PTPN22 | 1p13.3 | TCR and BCR signaling and other | RA, SLE,AITD, T1D |
| BAK1 | 4q22 | B cell activation/BCR signaling | SLE |
| TNF4/HP5 | 6q23 | Ubiquitin editing enzyme; inhibitor of TNFR signalling/NF-κB pathway | RA, SLE, CD |
| BLK | 8q23 | B cell activation | SLE |
| PTPN2 | 8p11.3 | Negative regulator of T cell activation | CD, T1D |
| TRAF1 | 9q33 | Regulates TNFR signalling/NF-κB pathway | RA |
| Intracellular pattern recognition receptors | | | |
| IFIH1 | 2q24 | Receptor for viral dsRNA | T1D, GD |
| NOQ2/ 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 |
| NFX2-3 | 10q24.2 | Regulates development of mesenterial and secondary lymphoid organs and B and T cell homing | CD |
| Cytokines and cytokine receptors | | | |
| IL2/IL21 | 4q36 | T cell regulation | T1D, RA, Celiac disease |
| IL23R | 1p31.1 | Th17 homeostasis | PSA, PSO, CD, AS |
| IL7RA | 3p13 | Memory T cell homeostasis | MS |
| IL2RA | 10p15.1 | T cell/Treg homeostasis | MS, T1D, GD |
| IL12B | 15q31.1 | Development of T cell subtypes, Th1 and Th17 | PSA, CD |
| Membrane receptors and co-stimulatory molecules | | | |
| CTLA4 | 2q33 | T cell costimulation inhibitor | T1D, RA |
| TLR6 | 16p11.2 | Immune complex clearance/leukocyte adhesion | SLE |
| CD40 | 20q12 | B/T cell costimulation | RA |
| Autophagy related | | | |
| ATG16L1 | 2q37.1 | Autophagy | CD |
| IRGM | 5q13.1 | Autophagy | CD |
| Enzymes | | | |
| ARTS1 | 5q15 | Peptide trimming for MHC I | AS |
| PASI4 | 1p36.13 | Enzymatic peptide catabolism | RA |
| Autoantigens | | | |
| IRS | 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 for autoimmunity.
- It remains unclear to what extent common variation versus multiple rare variants contribute to disease susceptibility.


PTPN2 risk alleles in Europe

Interplay between genes and environment in RA

Smoking causes citrullination of proteins in the lungs and the joints: antibodies to citrullinated 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

- HLA-DRB1 shared epitope alleles, PTPN22
- Smoking

ACPA-positive
- Higher disease activity
- More destruction
- More cardiovascular complications
- Higher all-case death rate

ACPA-negative
- Lower disease activity
- Lower all-case death rate
- Less destruction
- Fewer cardiovascular complications

Phenotype
- Same clinical presentation

Onset of disease
Putting everything together genes, environment and immune inflammatory response in RA
<table>
<thead>
<tr>
<th>Gene</th>
<th>Genome Location</th>
<th>Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA\†</td>
<td>6p21.33</td>
<td>Presentation of antigen</td>
</tr>
<tr>
<td>HLA\‡</td>
<td>6p21.32</td>
<td>Presentation of antigen</td>
</tr>
<tr>
<td>ITGAM\‡</td>
<td>16p11.2</td>
<td>Adhesion of leukocytes to endothelial cells</td>
</tr>
<tr>
<td>IRF5\‡</td>
<td>7q32.1</td>
<td>Production of interferon-α</td>
</tr>
<tr>
<td>KIAA1542\†</td>
<td>11p15.5</td>
<td>Linkage disequilibrium with IRF7; production of type I interferon</td>
</tr>
<tr>
<td>PXK\†</td>
<td>3p14.3</td>
<td>Unknown effect of serine–threonine kinase</td>
</tr>
<tr>
<td>PTPN22\†</td>
<td>1p13</td>
<td>Inhibition of lymphocyte activation</td>
</tr>
<tr>
<td>FCGR2A\†</td>
<td>1q23</td>
<td>Clearance of immune complexes</td>
</tr>
<tr>
<td>STAT4\‡</td>
<td>2q32</td>
<td>Modulation of the production of cytokines in T cells and natural killer cells;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>activation of response of macrophages to interferon-α</td>
</tr>
<tr>
<td>BLK\‡</td>
<td>8p23.1</td>
<td>Activation of B cells</td>
</tr>
</tbody>
</table>
Genes, environment 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 α in SLE

- Complement deficiencies may predispose to SLE

- The realization that the production of inflammatory cytokines including IFN-α may be mediated by endogenous ligands such as immune complexes
Modern genetics and ancient defenses in autoimmunity

**NALP1** in Vitiligo-Associated Multiple Autoimmune Disease


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

Jeanne Keffer, Lesley Probert, Haris Cazlaris, Spiros Georgopoulos, Evangelos Kaslaris, Dimitris Kioussis and George Kollias
Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis

Richard O. Williams*, Marc Feldmann, and Ravinder N. Maini
Inhibitors of co-stimulation
Anti-CD40L, CTLA-4Ig, Blys, Anti-LFA1

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

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 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
Microarray analysis reveals patient subgroups in the Bone Marrow
BM Genes differentially expressed according to disease activity

Granulopoiesis

Integrin signaling

Apoptosis of granulocytes
DNA microarrays studies identify only differentially expressed genes at the mRNA level.

Need to study gene regulation, protein levels, post-translational modifications and other regulatory mechanisms.
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
• 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).
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

<table>
<thead>
<tr>
<th>MicroRNA gene</th>
<th>Chromosomal location(^1)</th>
<th>Putative Targets(^2)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-150</td>
<td>19 : 54695854-54695937</td>
<td>c-myb</td>
<td>Myeloblastosis viral oncogene homolog</td>
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<td>hsa-miR-25</td>
<td>7 : 99529119-99529202</td>
<td>Bim</td>
<td>Bcl2-like 11</td>
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<td>hsa-miR-106b</td>
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<tr>
<td>hsa-miR-21</td>
<td>17 : 55273409-55273480</td>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
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<tr>
<td>hsa-let-7a</td>
<td>22 : 44887293-44887366</td>
<td>IL6</td>
<td>Interleukin 6</td>
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<tr>
<td>hsa-miR-196a</td>
<td>12 : 52671789-52671889</td>
<td>TCF7</td>
<td>Transcription factor 7, T-cell specific</td>
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<tr>
<td>hsa-miR-148b</td>
<td>12 : 53017267-53017365</td>
<td>DNMT3b</td>
<td>DNA Methyltransferase 3b</td>
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</tbody>
</table>

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

\(^2\)Putative microRNA targets according to prediction algorithms and expression data.

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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.
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 bionetworks

- SLE microRNA signature
- NHL microRNA signature
- hsa-miR-17-5p
- hsa-miR-150
- hsa-miR-26
- hsa-miR-155
- hsa-miR-21

Inhibition of apoptosis

Immune response

Systemic Lupus Erythematosus

DNA hypomethylation
PDCD4 in the immune system

  - Spontaneous lymphoma development in PDCD4 ko mice
  - PDCD4 deficient lymphocytes preferentially produce cytokines
Mir-21 is inversely correlated with PDCD4 in PBMCs of SLE patients.

**PD1 inhibition increases miR-21 expression**

**Proposed model for PDCD1 (PD1) regulating miR-21**

![Graph showing mRNA relative expression of miR-21 and PDCD4 in controls, SLE inactive, SLE active.](image)

![Western blot analysis showing PDCD4 and β-actin expression in Controls, SLE inactive, SLE active.](image)

**Diagram illustrating the proposed model:**
- miR-21 upregulation in SLE
- PDCD4 downregulation
- AP-1 activation
- 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

<table>
<thead>
<tr>
<th>ID</th>
<th>Score</th>
<th>Focus Molecules</th>
<th>Top Function</th>
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<tr>
<td>1</td>
<td>35</td>
<td>16</td>
<td>Cell Growth</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>14</td>
<td>Nucleic Acid Metabolism</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>12</td>
<td>Amino Acid Metabolism</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>6</td>
<td>Protein Synthesis</td>
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</table>

SLE active vs inactive patients

<table>
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<th>ID</th>
<th>Score</th>
<th>Focus Molecules</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>26</td>
<td>Energy Production</td>
</tr>
<tr>
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<td>47</td>
<td>25</td>
<td>Carcinogenesis</td>
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<tr>
<td>3</td>
<td>40</td>
<td>22</td>
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<td>35</td>
<td>20</td>
<td>Immune Response</td>
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<tr>
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<td>30</td>
<td>18</td>
<td>Cellular Growth and Proliferation</td>
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<td>6</td>
<td>28</td>
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<td>Protein Synthesis</td>
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<tr>
<td>7</td>
<td>26</td>
<td>16</td>
<td>RNA Post-Transcriptional Modification</td>
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<tr>
<td>8</td>
<td>18</td>
<td>12</td>
<td>Amino Acid Metabolism</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>10</td>
<td>Lipid Metabolism</td>
</tr>
</tbody>
</table>
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

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 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 of 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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