AUTOIMMUNITY

A major cause of morbidity and mortality in the world
Autoimmune diseases

• Systemic
  – Lupus
  – Sclerodermia
  – Vasculitis

• Organ-specific
  – Type 1 diabetes
  – Asthma
  – Rhumatoid arthritis
  – Myositis
  – Thyroiditis (Graves, Hashimoto)
  – APECED
  – Multiple sclerosis
  – Anemia, thrombopenia, neutropenia
  – Psoriasis, eczema
  – Gastritis
  – Colitis (Crohn, UC)
  – Myasthenia
  – Uveitis
  – Rhinitis
  –…..
Many autoimmune diseases are difficult or impossible to cure for the obvious reason that the focus of the immune response—self antigens—cannot be eliminated.

The ultimate mechanism involves a failure of tolerance and the accumulation of irreversible damage in the target tissues.
Proof of autoimmunity

- Damage in tissues due to immune cell infiltration
- Disease transferable by lymphocytes and/or serum
- Presence of cells/antibodies directed against self components
Scheme of immune cell function

Central Repertoire Selection

Homeostatic expansion

Peripheral pool

Half-life

DEATH

Self agonist selection

Secondary lymph organ

SELF TISSUE SURVEY/REPAIR

Innate cell priming

Memory

Regulatory activity

Antigen-driven expansion

Effector functions

Self TISSUE

SELF TISSUE

Survey/Repair

Death
Epidemiology

- Diseases affecting young adults
- Female > male
- Triggering and/or evolution often influenced by infectious diseases, pregnancy, traumatisms
- Familial trends
- North-South gradient
  - Higher prevalence in nordic countries
  - Inverse distribution with infectious diseases
Genetics

• Evaluate the degree of familial clustering
  • Concordance rates between monozygotic versus dizygotic twins
  • Increased risk of developing the disease in affected families

• Genetics of single gene disorders
  • Risk conferred to one individual is high but impact on the population is minimal due to the presence of rare variants
  • Deterministic relationship between the variant and the disease state

• Genetics of common autoimmune diseases
  • Common gene variants
  • Complex inheritance mode
  • Complex phenotypes
  • Likely overtime selection of gene variants by environment
Single gene disorders
AIRE and central tolerance

- Autoimmune polyendocrine syndrome (APS-1): a multiple attack against endocrine organs, skin and other tissues
- The AIRE gene controls the expression level of several promiscuously expressed antigens in the thymus
- Lack of AIRE leads to impaired tolerance by failure of central deletion
FOXP3 and regulatory T cells

- FOXP3, transcription factor of the forkhead family
- Lack of FoxP3 in mouse (scurfy mouse) leads to the absence of regulatory T cells
- Mouse equivalent of the rare human IPEX disease (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome)
FAS and lymphocyte apoptosis

- Fas (CD95), prototypic death receptor of the TNF family binds Fas-L
- Lpr/lpr and gld/gld strains of mouse share a common phenotype of lymphoproliferation and autoimmunity
- Lpr codes for Fas and gld for Fas ligand
- The APLS (autoimmune lymphoproliferative syndrome) disease mimics the mouse phenotype
- Fas contributes to the deletion of activated lymphocytes
Le modèle murin lpr / gld

Mutations de Fas (lpr) ou Fas-L

Lymphadénopathie et autoimmunité de type lupique

Homme: APLS

IL-2

Expansion  →  Mort

Tolérance périphérique par délétion Fas-médiiée
Déficit Fas / Fas-L et caspases

-> ALPS: Lymphoprolifération et Autoimmunité
Impact of single gene disorders on AI

Central Repertoire Selection

Homeostatic expansion

Peripheral pool

Half-life

Secondary lymph organ

Memory

Antigen-driven expansion

Death

FAS

AIRE

Self-agonist selection

Innate cell priming

Regulatory activity

Effector functions

FOXP3

SELF TISSUE SURVEY/REPAIR

SELF TISSUE

AIRE

FAS
Genetics of complex autoimmune diseases
Complex autoimmune diseases

- Chronic conditions elicited by a loss of immunological tolerance to self antigens

- Clinical manifestation: organ(s) failure

- Physiopathology: immune-mediated inflammatory disorder (IMID concept)

- 4-5% of the population, females > males

- Most common AIDs: Type 1 diabetes, Rhumatoid arthritis, lupus, Graves’ disease, multiple sclerosis, pernicious anemia

- 1 in 30 individuals affected: major health problem (2 x cancer ?)
Impact of complex gene disorders on AI

- Central Repertoire Selection
- Homeostatic expansion
- Peripheral pool
- Half-life
- Innate cell priming
- Secondary lymph organ
- Memory
- Effector functions
- Antigen-driven expansion
- Regulatory activity
- Self-agonist selection
- SELF TISSUE SURVEY/REPAIR
- SELF TISSUE
Strategy for analysis

• 1. Defining immune phenotypes
• 2. Phenotype / Genotype linking
• 3. Defining gene polymorphism
• 4. Identifying pathogenic mechanisms
• 5. Defining epistatic interactions (additive / synergistic / suppressive)
• 6. Reconstituting the pathology
Immune features in AID

- **Autoreactivity**
  - Autoantibodies: RA, MG, SLE
  - T lymphocytes: T1D, Thyroiditis
  - Altered regulation: IPEX, APECED

- **Cell activation**
  - Innate immune cell activation
  - Lymphocyte hyper/hypo activation: SLE
  - Inflammation (C’, …): Pemphigus

- **Homeostatic phenotypes**
  - Cell death: T1D, SLE
  - Microenvironmental alterations: SLE

- Various AID display at various levels these phenotypes
- Some are responsible for the pathogenesis, others are associated to it, few are specific for a given pathology
Linkage versus association studies
Linkage studies

- **Inheritance** of AID susceptibility is complex
- **MHC** exerts a predominant influence
- Many genomic segments show weak statistical association (lod scores from 2-5 versus 30 for a fully penetrant Mendelian disease locus)
- **Multifactorial diseases** result from the combined impact of multiple susceptibility genes, further enriched by poorly defined environmental factors

- Complex but chronic so in principle accessible to therapeutic interventions if **predictions** are reliable
## Family studies in human AIDs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Concordance rate (%)</th>
<th>Population prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monozygotic twins</td>
<td>Dizygotic twins</td>
</tr>
<tr>
<td>( \lambda_s ) 10-40</td>
<td>30-50</td>
<td>0-13</td>
</tr>
<tr>
<td>Diabetes</td>
<td>30-50</td>
<td>0-13</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>25</td>
<td>0-5</td>
</tr>
<tr>
<td>Lupus</td>
<td>24-57</td>
<td>2-5</td>
</tr>
<tr>
<td>Rhumatoid arthritis</td>
<td>12-15</td>
<td>3-4</td>
</tr>
</tbody>
</table>

Multigenic

Genetic influence
Linkage to MHC alleles

• Suggestive of T cell mediated dys-immunity

• Suggestive of a restricted set of primary autoantigens (still ill-defined)

• Existence of MHC molecules with peculiar antigen-binding properties

• Peptide register shifting in MHC class II groove renders the predictive task more difficult
Difficulty of linkage analysis

- Most MHC-linked diseases are multigenic

- Strong linkage disequilibrium in the MHC locus
Backcrosses in mouse

Transgenes are inserted into F2 embryos

(AxB)F1 ♀ × ♂

The resulting offspring have a heterogeneous genetic background

Backcross transgenic animal to strain of interest, e.g., C57BL/6

B6 mouse transgenic

Continue backcross for 10–20 generations

B6 mouse

Assess functions of transgenes in homogenous genetic background
Single Nucleotide Polymorphism SNP

- Most abundant form of DNA variation
- Around 7 million SNPs with MAF around 5% (minor allele frequency) and 4 million SNPs with MAF between 1 and 5%, plus numerous rare SNPs
- Relation between common variants and human phenotypes (height, eye color, disease susceptibility) not known
- Global approach for a dense SNP map: oligonucleotide arrays
- Hinds et al (Science 2005): 1.6 million SNPs mapped covering 95% of the genome (inter-SNP intervals < 50kB) -> informative of population ancestry, but in agreement with the notion that most common DNA variations are shared across human populations
Penetrance of susceptibility genes

• Basic observation: discordance rate between monozygotic twins => incomplete penetrance of susceptibility genes

• Environmental triggers (difficult to define !)

• Intrinsic parameters: parental imprinting, allelic exclusion of BCR and TCR, monoallelic expression of cytokines, NKR

• Importance of other non-MHC genetic loci
TYPE 1 DIABETES

An autoimmune disease
Human Type 1 diabetes

- Autoimmune disease affecting 0.5% of the population
- Incidence in Western world has been doubling every 15 years since the last 50 years
- Due to the destruction of insulin-producing islet Langerhans beta cells in the pancreas
- Both genetic and environmental factors are involved
- Physiopathology: failure to use glucose in tissues leads to hyperglycemia and downstream complications
Histopathology of islet beta cells
Environmental versus genetic factors

• Genetically identical human twins: less than 40% concordance

• Putative link with exposure to cow milk proteins during early infancy?

• Association of diabetes with infections:
  - Enhanced diabetes incidence in NOD mouse colony housed in a clean animal facility
  - Periodical onset of diabetes in humans: cosxackie B4, ...
  - Inverse relationship between increased hygiena and diabetes in western world
Viral infections and diabetes

• A difficult link to establish
  - Multiple viral infections in life prior to diabetes onset
  - Virus may be cleared prior to autoimmune attack
  - Coxsakie viruses are still suspect

• Putative provoking / enhancing mechanisms
  - Local inflammation induces recruitment and/or bystander activation
  - Molecular mimicry
  - Release of sequestered antigens -> epitope spreading

• Protective infections (EMCD virus, induction of Th2 responses by Mycobacterial hsp65 injection)
Genetic predisposition

- Twin studies: concordance rate < 40% (risk higher in monozygotic / dizygotic twins)

- Diabetes frequency is higher amongst genetically related family members (6% versus 0.5% in the population)

- Presence of autoantibodies in prediabetic patients and close relatives

- Linkage analyses among families and association analyses identified susceptibility loci (> 20)

- The HLA locus accounts for 50% of the genetic risk

- Other loci have weak effects taken individually and may affect various phases of the autoimmune process
MHC: a central player

<table>
<thead>
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</tr>
<tr>
<td>Diabetes</td>
<td>30-50</td>
<td>0-13</td>
</tr>
</tbody>
</table>

Other genes

MHC weight

MHC identical siblings

15%
## Conserved Extended Haplotypes

<table>
<thead>
<tr>
<th>CEH type</th>
<th>CEHs [HLA-B, complotype, DR]</th>
<th>Ratio (T1D:normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptibility</td>
<td>B62, SC31, DR4, DQ8</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>B18, F1C30, DR3, DQ2</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>B62, SB42, DR4</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>B62, SC33, DR4</td>
<td>4.4</td>
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<td></td>
<td>B8, SC01, DR3</td>
<td>2.1</td>
</tr>
<tr>
<td>Neutral</td>
<td>B60, SC02, DR6</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>B35, FC(3,2)0, DR1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>B44, SC30, DR4</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>B35, SC31, DR5</td>
<td>0.45</td>
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<tr>
<td>Protective</td>
<td>B7, SC31, DR2</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>B44, FC31, DR7</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>B57, SC61, DR7</td>
<td>0.14</td>
</tr>
</tbody>
</table>
MHC susceptibility locus for T1D

- **Frequency** of MHC susceptibility alleles in Caucasian population: 53% => More frequent than protective alleles !!
- Mouse equivalent: I-A\(^g_7\) (Tg allo-MHC protective)

- **Inheritance**
  - Recessive mode for susceptibility
  - Dominant mode for protection

- Incomplete **penetrance** of susceptibility alleles

- Impact of susceptibility alleles on Ag binding
Genetic complexity and heterogeneity

• Association studies differ between ethnic groups
• Genetic localisations of AIDs vary between disease models

T1D red
MS blue
RA green
Non HLA associated loci in T1D
# Linkage data in T1D

<table>
<thead>
<tr>
<th>Chromosomal Region</th>
<th>Position (cM)</th>
<th>Closest Marker</th>
<th>Lod Score</th>
<th>Sibling Risk Ratio</th>
<th>Lod-1 Interval†</th>
<th>Nominal P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2q31-33</td>
<td>192</td>
<td>D2S2167</td>
<td>3.34</td>
<td>1.19</td>
<td>177–204</td>
<td>9.0×10⁻⁵</td>
</tr>
<tr>
<td>3p13-p14</td>
<td>98</td>
<td>D3S1261</td>
<td>1.52</td>
<td>1.15</td>
<td>78–112</td>
<td>8.2×10⁻³</td>
</tr>
<tr>
<td>6p21</td>
<td>47</td>
<td>TNFA</td>
<td>116.3</td>
<td>3.35</td>
<td>46–48</td>
<td>4.9×10⁻⁵²</td>
</tr>
<tr>
<td>6q21</td>
<td>80</td>
<td>D6S283</td>
<td>22.39</td>
<td>1.56</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9q33-q34</td>
<td>150</td>
<td>D9S260</td>
<td>2.20</td>
<td>1.13</td>
<td>138–161</td>
<td>1.5×10⁻³</td>
</tr>
<tr>
<td>10p14-q11</td>
<td>61</td>
<td>D10S1426</td>
<td>3.21</td>
<td>1.12</td>
<td>52–66</td>
<td>1.2×10⁻⁴</td>
</tr>
<tr>
<td>11p15</td>
<td>2</td>
<td>D11S922</td>
<td>1.87</td>
<td>1.16</td>
<td>0–14</td>
<td>3.4×10⁻³</td>
</tr>
<tr>
<td>12q14-q12</td>
<td>81</td>
<td>D12S375</td>
<td>1.66</td>
<td>1.10</td>
<td>77–83</td>
<td>5.8×10⁻³</td>
</tr>
<tr>
<td>16p12-q11.1</td>
<td>56</td>
<td>D16S3131</td>
<td>1.88</td>
<td>1.17</td>
<td>26–71</td>
<td>3.3×10⁻³</td>
</tr>
<tr>
<td>16q22-q24</td>
<td>108</td>
<td>D16S504</td>
<td>2.64</td>
<td>1.19</td>
<td>100–121</td>
<td>4.9×10⁻⁴</td>
</tr>
<tr>
<td>19p13.3-p13.2</td>
<td>25</td>
<td>INSR</td>
<td>1.92</td>
<td>1.15</td>
<td>0–43</td>
<td>3.0×10⁻³</td>
</tr>
</tbody>
</table>

* Data are from the Type 1 Diabetes Genetics Consortium. The abbreviation cM denotes centimorgan, lod logarithmic odds, and ND not done.
† The lod-1 interval is the size of the interval (in centimorgans) in which the lod score is greater than or equal to the maximum minus 1.0.
Autoimmunity in type 1 diabetes

- Islet beta cells
- Autoantigen release
  - Priming
  - Autoimmune lymphoid repertoire
  - Recruitment
  - Expansion
  - Differentiation
  - Effectors
  - Regulation
  - Loss of tolerance

TIME
The NOD mouse model of T1D

- Human T1D
  - No sex bias
  - Onset in young people
  - MHC linkage (DR/DQ)
  - Multigenic
  - Environmental factors
  - T cell mediated autoimmunity

- NOD mouse
  - Predominant in female
  - Early onset
  - I-Ag7, DQ8 equivalent, no DR
  - Multigenic
  - Environmental factors
  - T cell mediated autoimmunity
  - Lack of complement C5
Diabetes in NOD mouse

- 80% incidence in female mice between 12 and 30 weeks of age

- Progression through two checkpoints
  - Before 3 wks, onset of insulitis (APC composition, homing processes)
  - Around 8-12 wks, destructive insulitis (imbalanced cell composition)
Search for autoantigens

Auto-immunisation

3 étapes essentielles

- constitution d’un répertoire auto-réactif

- recrutement et activation sur une cible focalisée

- dysfonctionnement des mécanismes régulateurs de l’auto-réactivité en périphérie
Initial pancreatic insult

- Provoking beta cell death: physiological ripple of death in mice at 2 wks or STZ-induced cell death
- Dead cells are taken up by pancreatic DC and transported to PLN
- Attracting antigen-specific lymphocytes (not NOD dependent) after 2-3 wks requires altered APC functions
- An augmented autoreactive T cell repertoire might be characteristics of the NOD background
- Infections could modulate this initial step
- Nicotinamide protects from diabetes by inhibiting NO production
Autoantibodies are early markers of disease progression

- Antibodies to insulin, GAD, insulin granule membrane proteins (ICA and phogrin IA2β) are detected prior to disease onset

- They are reliable markers of diabetes predisposition in HLA-prone individuals

- Autoantibodies to non pancreatic antigens are found (hsp, thyroid antigens, …)
T lymphocytes are pathogenic

- Islets are infiltrated with T cells
- Thymectomy prevents diabetes onset
- NOD-SCID mice are not diabetic
- Transplantation of islets in thymus abrogates diabetes
- Disease is transferable by T cell populations or clones
- Both CD4 and CD8 T lymphocytes are required, thus both class II and class I MHC restricted peptides should be identified
MHC contribution to peptide recognition

- NOD I-\(\text{A}^b\) or B6 H-\(2^g7\) mice do not develop diabetes

- Selection of an autoimmune repertoire is strictly MHC-dependent

- Model system: BDC2-5 is an autoreactive CD4+ T cell clone
  - Using MHC tetramer with high affinity for the BDC2-5 TCR, one can show that the selection and thymic export of BDC2-5 cells is only MHC dependent
  - Thus, susceptible MHC alleles and not background genes select an autoimmune reactivity
  - Contradictory argument: graft of NOD thymic epithelium rudiment might preset T repertoire for autoimmunity?
HLA = *IDDM1*

- **Class II MHC** > **class I MHC**

- *Alleles are associated with risk (DR3 / DR4, DQb1 asp57) or protection (DR2, DR6)*

- *But strong linkage disequilibrium prevents refined studies*

- *Diabetogenic MHC poorly binds autoantigens → impaired thymic deletion*
Is there a primary autoantigen?

- Its recognition should occur early and could be dependent upon expression of susceptibility MHC alleles
- Its absence should prevent or delay diabetes onset
- Its pattern of expression should be restricted to a peripheral tissue
- Tolerance to this molecule should rely on ignorance
- Tolerance failure probably involves a local triggering lesion
- It doesn’t need to be a major target for tissue destruction but could activate effector (Th1 ?) cells to boost inflammation
Insulin locus = *IDDM2*

- Association between T1D and VNTR markers in the insulin promoter
- Affects intrathymic insulin gene expression and thus repertoire selection
- Insulin 2 KO mouse has reduced thymic insulin expression and increased diabetes incidence
Preproinsulin 1 is a good candidate

- In NOD mice, two insulin genes exist with distinct promoters
- Insulin 1 KO mice bred on the NOD background do not develop insulitis and diabetes
- In contrast, GAD 65 KO mice still develop diabetes
- Insulin is not the only antigenic target: ins 1 KO islets transplanted in diabetic NOD mice are rejected
- The B chain epitope B9-23 binds I-A\(^g\) and triggers autoreactive CD4\(^+\) cells which transfer diabetes
- A linked epitope (B24-C36) binds K\(^d\) and activates CD8\(^+\) T cells
- Recessive tolerance induction using insulin peptides has been shown
Immunodominant beta cells autoantigens

- Proinsulin / insulin (B9-23) : HLA DQ8
- GAD65 (271-285) : HLA DR 0401
- Hsp 60
- ICA antigens (phogrin, ICA251)
Autoantigens for CD8+ cells

- CD8+ T cells are required for diabetes

- In NOD mice lacking islet cell MHC class 1 expression, initiation and progression of diabetes is unperturbed but mice never become hyperglycemic

- Several antigens are recognized: insulin, a pancreatic homolog of glucose 6 phosphatase (IGRP)

- The IGRP gene maps close to IDDM7

- Possible molecular mimicry with a Borrelia Burgdorferi peptide, responsible for arthritis reactions in Lyme disease
T cell repertoire in NOD mice

- Impaired negative selection in NOD mice
- Transplantation of a NOD thymic epithelium controls diabetes onset
- Expression of pancreatic autoantigens in thymus is associated with tolerance to insulin (IDDM2 locus)
Central tolerance to insulin?

- The insulin 2 gene is weakly transcribed in thymic medullary epithelial cells; in human, polymorphism in the insulin promoter affects thymic expression levels.

- Insulin gene expression is promiscuous and depends on the AIRE transcription factor in thymus.

- AIRE is a major controller of central tolerance to peripheral self antigens.

- AIRE KO mice show pancreatic infiltrates and some APECED patients have T1D.
Effector mechanisms in T1D

Checkpoints

- Peri Insulitis (4-6 weeks)
  - self-antigen primed T cells CD4+/CD8+, B cells
  - regulatory T cells
  - monocytes

- Insulitis (15-20 weeks)
  - proinflammatory cytokines (IL-18, IL-12, TNFα, IL-1β, IFNγ)
  - infiltrating effector cells
The Idd3 locus contains the IL-2 gene

- No definitive proof that IL-2 is involved
- Requires KI replacing the NOD IL-2 gene by a protective variant
- Possible impact on IL-2 biodelivery / glycosylation?
Cbl-b mutation in a rat model of T1D

- Cbl-b is a negative regulator of T cell activation (ubiquitine ligase activity regulating requirement for costimulus)

- Lack of cbl-b bypasses the need for signal 2 and provokes devastating autoimmunity

- Identification of a mutation responsible for T1D in rat
CTLA4 = IDDM12

- Identified by positional candidate gene mapping
- Clustering of different autoimmune diseases in families suggest common genetic background (T1D, thyroiditis)
- Locus contains CD28, CTLA-4, ICOS
- Sequence -> 1 SNP in the 3’UTR of CTLA-4 -> controls splicing efficiency and production of soluble CTLA-4 isoform which inhibits T cell activation
- Susceptibility allele leads to less sCTLA-4, maybe selected in the context of infectious diseases over evolution
PTPN22

• Intracellular phosphatase in lymphocytes

• Interaction with CSK

• Dephosphorylation of Ick, ZAP 70, VAV, CD3z

• Quantitative trait locus in T1D?
Islet cell death

• Clinical symptoms > 70% islet cell death
• Mediators / mechanisms
  – Fas / Fas-L: induction by IFN / IL-1b, delayed onset if DN Fas in beta cells (FADD)
  – Perforin and granzyme: KO perforin -> insulitis without diabetes
  – Inflammatory cytokines: IFNγ / IL-1β / TNF
  – NO -> ER-stress -> islet cells more sensitive to ER stress
  – Lipo and glucotoxicity
PTPN2

- Induced by IFNg in islets
- Inhibits cytokine-induced cytotoxicity of islet cells
- Phosphatase activity reducing STAT1 activation on the IFNg pathway
Linked susceptibility alleles

- Ex: NOD Idd9 contains molecular variants of CD30, Tnfr2 and CD137

- The MHC locus contains both MHC and non-MHC susceptibility genes: maybe TNF or the proteasome subunits?

- Linked alleles might have been coselected through evolution

- Detection of susceptibility loci with moderate effects using classical association analysis might preferentially detect linked common allelic variants

- These variations are likely to affect immune responses: back to the concept of « Ir genes »
Paradoxical protective NOD alleles

- Use BDC2.5 islet-specific TCR transgenic T cells to bypass diabetogenic repertoire amplification (reducing complexity)
- Diabetes onset is frequent and early in B6<sup>g7</sup> but not in NOD mice
- The B6 phenotype is associated with early insulitis
- Backcross on B6<sup>g7</sup> shows rapid loss of diabetes penetrance suggesting that few gene loci contribute to the B6 phenotype
Genetic background: Th1 / Th2

- RIP-HA x TCR anti-HA double Tg mice
- Diabetes on B6 and not BALB/c background
- Backcross on B10.D2 leads to the rapid loss of dominant resistant alleles and diabetes appearance
- Resistance is linked to the Th2 bias in the BALB/c mouse background
Differential roles of risk loci in T1D
## Diabetes incidence in mutant NOD mice

<table>
<thead>
<tr>
<th>Antibody/Marker</th>
<th>Changes</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28</td>
<td>augmented</td>
<td>Less CD25+ T reg</td>
</tr>
<tr>
<td>B7</td>
<td>augmented</td>
<td>Less CD25+ T reg</td>
</tr>
<tr>
<td>CD54 (ICAM-1)</td>
<td>abolished</td>
<td>Reduced trafficking</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Generalized autoimmunity</td>
<td>Lack of negative regulation</td>
</tr>
<tr>
<td>IL-4</td>
<td>unchanged</td>
<td>Th2 failure</td>
</tr>
<tr>
<td>γIFN</td>
<td>delayed</td>
<td>Reduced Th1 responses</td>
</tr>
<tr>
<td>IL-12</td>
<td>unchanged</td>
<td></td>
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</tbody>
</table>
### Effect of cytokines on diabetes incidence

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Effect</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic IL-2</td>
<td>enhanced</td>
<td>Breaks tolerance?</td>
</tr>
<tr>
<td>IL-4</td>
<td>abolished</td>
<td>Inhibits Th1</td>
</tr>
<tr>
<td>Pancreatic IL-10</td>
<td>enhanced</td>
<td>B-cell dependent antigenic spreading?</td>
</tr>
<tr>
<td>TNF early</td>
<td>enhanced</td>
<td>Increased inflammation</td>
</tr>
<tr>
<td>TNF late</td>
<td>abolished</td>
<td>Death of autoreactive cells</td>
</tr>
</tbody>
</table>
Diversification antigénique

Lésion initiale

Quelques auto-Ag
voire 1 ?

Quelques auto-Ag
voire 1 ?

Plus tard

Nombreux auto-Ag

B = CPA x 1000

Présentation
d ’Ag minoritaires

Anticorps / BCR
= concentrateur d ’Ag

Lésion initiale

Quelques auto-Ag
voire 1 ?

Plus tard

Nombreux auto-Ag

B = CPA x 1000

Présentation
d ’Ag minoritaires

Anticorps / BCR
= concentrateur d ’Ag
Inhibitory cytokines

- **IL-10**: acts mainly via APC by inhibition of APC-dependent T cell activation
- **TGFβ**: acts at different levels but reduces T cell activation and primes Treg
- **IL-4**: contributes to immune deviation from Th1 to Th2 protective cells
Regulatory function of IL-10

• BDC2-5 transgenic mice show a low incidence of diabetes

• Adoptive transfer from young but not old BDC2-5 Tg mouse provokes diabetes

• Anti-IL-10R mAb reverses a dominant inhibitory effect

• Thus IL-10 participates to the maintenance of a dominant tolerance in this transgenic mouse model; this probably involves regulatory T cells belonging to the non transgenic T cells
Altering self tolerance

- Cyclophosphamide treatment alters T cell regulation and provokes diabetes
- Anti TGFb antibodies abrogate the protective capacity of regulatory T cells
- Transgenic expression of pancreatic TNF and CD80
Restoring self tolerance

• Diabetes: Th1 excess, Treg defect?

• CD4+CD25+ from NOD mice have reduced suppressive function in vitro; this phenotype progressively develops in NOD mice

• Treatment with anti-CD3 mAb in vivo restores Treg numbers, as well as in vitro and in vivo regulatory potential
  - This involves TGFβ and not IL-4 or IL-10 production and requires CTLA4 engagement

• Transgenic expression of activated Notch3 augments Treg numbers and protects from streptozotocine induced diabetes

• Modulating islet cell apoptosis
Controlled islet cell apoptosis reduces diabetes onset in NOD mice

• Low doses of STZ injected in NOD mice prior to diabetes onset reduce disease incidence

• Apoptosis is required since transgenic expression of Crma in islets prevents this effect

• T cells from STZ-treated NOD mice fail to transfer disease

• Evidence for emergence of regulatory T cells
NKT cells

- Reduced numbers in NOD mice
- Injection of NKT cells protects from diabetes
- Activating NKT cell ligands block diabetes (αGal Cer) in vivo
- NKT cells negatively regulate Th1 functions
- NKT cell deficiency linked to Idd locus in mouse but is not detected in human T1D
Restoring antigen-specific tolerance

- Injection of soluble autoantigens
- Intrathymic injection
- Transfer of proinsulin encoding hematopoietic stem cells
- Inducing bystander immunosuppression using insulin B chain - specific regulatory T cells (IL-4 dependent in vivo?)
- Immune deviation/anergy can be induced by soluble MHC-HA peptide complexes in double Tg mic expressing HA in pancreas and housing HA-specific Tg T cells
Multiple immunological defects in type 1 diabetes

Islet beta cells

Autoantigen expression
GAD, insulin, IA, ...

Peri-insulitis
Autoimmune lymphoid repertoire

STRESS

Insulitis

Recruitment
Chemokines

Expansion

Priming

HLA

Differentiation

Regulation

Diabetes

CD4+, Th1 > Th2
Regulatory T/NKT?

CD8+, B, CPA
Therapeutic approaches

- **Global strategies**
  - Immunosuppression (CSA)
  - Cytokines: IL-4, IL-10, IL-11, IL-13, TGFβ
  - Antibodies to γIFN, CD4, CD8, CD3, CD40L

- **Antigen-specific strategies**
  - Soluble antigens (insulin, GAD, hsp peptides) but short half life in vivo
  - Priming Ag-specific T regs (low dose of STZ-induced beta cell apoptosis)
  - Engineered peptide / MHC complexes
Le Lupus Érythémateux Disséminé

Manifestations cliniques et démarche diagnostique chez l’homme

Dr Nicolas SCHLEINITZ
Service de Médecine Interne Pr JR Harlé
CHU Conception
Plan du cours

Le lupus érythémateux disséminé

1- Epidémiologie

2- Les manifestations cliniques et biologiques

3- Les traitements
### Épidémiologie

<table>
<thead>
<tr>
<th>Incidence</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasiens hommes</td>
<td>0.3 à 0.9 / 100.000 hab</td>
</tr>
<tr>
<td>Caucasiens femmes</td>
<td>2.5 à 3.9 / 100.000 hab</td>
</tr>
<tr>
<td>African Americans hommes</td>
<td>0.7 à 2.5 / 100.000 hab</td>
</tr>
<tr>
<td>African Americans femmes</td>
<td>8.1 à 10.4 / 100.000 hab</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prévalence</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasiens femmes</td>
<td>17 à 71 / 100.000 hab</td>
</tr>
<tr>
<td>African Americans femmes</td>
<td>56 à 283 / 100.000 hab</td>
</tr>
</tbody>
</table>
Épidémiologie

Terrain génétique prédisposant

Influence du sexe (facteurs hormonaux?)

8 femmes / 1 homme

(2F/1H prépuberté et après ménopause)

Age de début: 10 à 40 ans (30 ans en moyenne)
Le lupus érythémateux disséminé

Critères diagnostiques ACR

1. Érythème malaire
2. Lupus discoïde
3. Photosensibilité
4. Ulcérations orales
5. Arthrite
6. Atteinte séreuse
7. Atteinte rénale
8. Atteinte neurologique
9. Atteinte hématologique
10. Atteinte immunologique :
    - anticorps anti-ADN natif à un titre anormal ;
    - anticorps anti-Sm ;
    - présence d’anticorps antiphospholipides correspondant soit à : 1) un taux élevé d’anticorps anticardiolipine de type IgG ou IgM ; 2) un anticoagulant de type lupique ; 3) une sérologie syphilitique dissociée depuis plus de 6 mois.
11. Anticorps antinucléaires
Il suffit de 4 critères sur 11 pour retenir le diagnostique

Pourquoi des critères ?
Critère N°1: Le rash malaire 
(Aspect en loup)
La biopsie cutanée montre une destruction de la membrane basale (1), un infiltrat inflammatoire (2), et une extravasation de globules rouges (3).
L’analyse en IF avec un anti-IgG retrouve une « bande lupique » : dépots d’IgG le long de la membrane basale.
Critère N°2:  
Le lupus discoïde
Critère N°3: Photosensibilité

Lésions cutanées anormales au niveau des zones exposées
Critère N°4: Les ulcérations buccales et/ou nasales
Critère N°5: Arthrite

Arthrite symétrique

Touche surtout les articulations périphériques
Pannus synovial
Inflammation et épaississement de la synovie
Critère N°6: Atteinte séreuse

Péricardite

Pleurésie
Critère N°7: Atteinte rénale

6 types histologiques (mais formes de passage)
- Classe I = rein normal, IF +
- Classe II (25 %) = GN mesangiale, IF +
- Classe III (15 %) = GN segmentaire et focale
- Classe IV (30 à 40 %) = GN proliférative diffuse
- Classe V = GN extramembraneuse
- Classe VI = GN avec sclérose avancée
Biopsie rénale

Dépots de C1q

Dépots d’IgG
Biopsie rénale

- **classe II**
- **classes III-IV**
- **classes VI**

**Prolifération cellulaires**

**Insuffisance rénale terminale**

**Dialyse ou greffe rénale**
Critère N°8: Atteinte neurologique

- Epilepsie
- Psychose
- Maladie démyélinisante

Liée à une vascularite cérébrale
Vascularite = inflammation des vaisseaux

Responsable d’ischémie
Critère N°9: Atteinte hématologique

- Thrombopénie
- Anémie hémolytique
- Leucopénie

Auto-anticorps dirigés contre des protéines membranaires des Plaquettes et/ou des GR
Critère N°10: Atteinte Immunologique

10. Atteinte immunologique :
- anticorps anti-ADN natif à un titre anormal ;
- anticorps anti-Srn ;
- présence d'anticorps antiphospholipides correspondant soit à : 1) un taux élevé d'anticorps anticardi olipine de type IgG ou IgM ; 2) un anticoagulant de type lupique ; 3) une sérologie syphilitique dissociée depuis plus de 6 mois.

11. Anticorps antinucléaires
Critère N°10: Anticorps anti-nucléaires

Recherchés par IFindirecte
Critère N°10: Anticorps anti-DNA natif

Ac anti *DNA natif* dirigés contre l’ADN double brin « Spécifiques » du LED et « corrélés » à l’activité de la Maladie

Ac anti *DNA dénaturé* dirigés contre l’ADN simple brin (lupus induit)
Les autres anomalies biologiques observées dans le cadre du LED:

Hypergammaglobulinémie polyclonale = activation B polyclonale

Effondrement des protéines du complément au dosage sérique = consommation du complément par la formation de complexes immuns (signe biologique d’activité de la maladie)

Augmentation des protéines de phase aigue de l’inflammation
Le lupus est une maladie auto-immune qui peut toucher tous les organes = maladie systémique

Elle peut s’associer dans certains cas à des auto-anticorps qui reconnaissent des phospholipides membranaires et peuvent entraîner un état d’hypercoagulabilité : il s’agit du syndrome des antiphospholipides.
Syndrome des Antiphospholipides

Un critère clinique :
Un épisode thrombotique *veineux ou artériel*
*ou*
Une complication obstétricale (Avortements précoces répétés, Fausse couche tardive, pré-éclampsie)

Un critère biologique :
Présence d’un anticoagulant circulant de type lupique *ou*
Présence d’anticorps anticardiolipines (retrouvés positifs deux fois à au moins six semaines d’intervalle)
Infarctus mésentérique
Infarctus splénique
Autoantibodies in SLE

- Antinuclear Ab
  - Sensitive test for SLE
  - Not SLE specific
  - Not associated with severity of SLE (flares without ANA)
  - High ANA levels in the absence of disease
- Anti-Sm Ab specific for SLE
- Anti-RNP Ab associated with subset of patients with mixed connective tissue disease
- Anti-phospholipid Ab predisposition to thrombotic events
- Anti-ribosomal P Ab increased risk of CNS disease
- Anti-Ro (SSA) Ab increased risk of heart block in newborns of SLE patients
La définition médicale du LED repose donc sur un ensemble de manifestations cliniques +/- associées et l’existence de marqueurs biologiques.

Il s’agit d’une maladie **chronique** évoluant par **poussées** pour laquelle il n’existe aucun traitement spécifique à l’heure actuelle.

Ces manifestations sont la conséquence d’une **réaction inflammatoire et auto-immune inadaptée**.

Sans traitement l’espérance de vie est réduite actuellement la survie à 10 ans est > 80%.
Traitement du lupus

Des traitements non spécifiques avec des effets secondaires à long terme.
Décès à long terme : Lupus < Pathologies cardiovasculaires
Pattern de mortalité bi-modal:
lupus au début / pathologies cardiovasculaires plus tard

Le traitement est adapté aux manifestations cliniques
Traitement du lupus

**Traitements anti-inflammatoires: La corticothérapie**

**Traitements immuno-suppresseurs: L’azathiprine, le Methotrexate, le Cyclophosphamide, le Mycophenolate mofetil, la ciclosporine...**

**Traitements immunomodulateurs: les Ig par voie intraveineuse, le plaquenil**
Traitement du lupus

Les traitements plus ciblés:

Utilisation d’anticorps monoclonaux à visée thérapeutique:

Terrain génétique prédisposant
Facteurs hormonaux
Facteurs environnementaux
Infections

Réponse immunitaire adaptative

Production d’auto-anticorps spécifiques de tissus

Formation de complexes immuns => vascularite et atteinte rénale
Systemic lupus erythematosus

Physiopathology
Spontaneous murine models of SLE

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>NZB</th>
<th>(NZB×NZW)F1</th>
<th>MRL</th>
<th>BXSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Mortality (month)</td>
<td>14 (F/M)</td>
<td>9 (F)/16 (M)</td>
<td>5 (lpr/lpr)/17 (+/+)</td>
<td>6 (M)/20 (F)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Arthritis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Anti-DNA</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Anti-RBC</td>
<td>+++</td>
<td>+</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>Anti-IgG</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

-> Analysis of phenotypes
Candidate susceptibility genes

- HLA: DR-B1 (DR2, DR3)
- Genes of the HLA region in linkage disequilibrium: MHC class III (TNF, TAP, HSP)
- FcγR: low affinity receptor for IgG and IgG IC
- Complement: classical pathway
### Genetic association studies of systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>Gene</th>
<th>OMIM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cytogenetic location</th>
<th>Associated allele(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcGR2A</td>
<td>146790</td>
<td>1q22-23</td>
<td>R131</td>
</tr>
<tr>
<td>FcGR3A</td>
<td>146740</td>
<td>1q22-23</td>
<td>F176</td>
</tr>
<tr>
<td>IL10</td>
<td>124092</td>
<td>1q31-32</td>
<td>Multiple alleles</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>123890</td>
<td>2q33</td>
<td>+49G</td>
</tr>
<tr>
<td>PDCD-1</td>
<td>600244</td>
<td>2q37</td>
<td>PD-1.3A</td>
</tr>
<tr>
<td>HLA-DR3, -DR2</td>
<td>152700 142860</td>
<td>6p21</td>
<td>DR2/DR3</td>
</tr>
<tr>
<td>TNFα</td>
<td>191160</td>
<td>6p21</td>
<td>TNF2</td>
</tr>
<tr>
<td>TNFβ</td>
<td>153440</td>
<td>6p21</td>
<td>TNFB*2</td>
</tr>
<tr>
<td>C4</td>
<td>120790</td>
<td>6p21</td>
<td>AQ0</td>
</tr>
<tr>
<td>MBL</td>
<td>154545</td>
<td>10q11.2-q21</td>
<td>230A</td>
</tr>
<tr>
<td>FASL</td>
<td>134637</td>
<td>1q23</td>
<td>−844C</td>
</tr>
<tr>
<td>FAS</td>
<td>134638</td>
<td>10q24</td>
<td>297C/416G</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>151430</td>
<td>18q21</td>
<td>Multiple alleles</td>
</tr>
</tbody>
</table>
Examples of genetic manipulations that lead to lupus-like disease in mice.

<table>
<thead>
<tr>
<th>Targeted gene producta</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fas, Fas ligand, Bcl-2 (Tg), IEX-1(Tg)</td>
<td>Defective apoptosis</td>
</tr>
<tr>
<td>SAP, DNase, Mer, MFG-E8, IgM (secreted), C1q, C4</td>
<td>Defective clearance of apoptotic cells, cell debris, and/or ICs</td>
</tr>
<tr>
<td>FcyRIIB, TACI, CD22 TGFβRII, CD45*, PD-1 BAFF (Tg)</td>
<td>Dysregulated lymphocyte activation caused by receptors and their ligands</td>
</tr>
<tr>
<td>SHP-1, Lyn, PKC-d, P21, E2F2, Stra13, Gadd45a, Rasgrp1, Fli-1 (Tg), SOCS-1, LIGHT (Tg) TSAd</td>
<td>Dysregulated lymphocyte activation caused by intracellular signaling molecule mutations</td>
</tr>
<tr>
<td>TGFβ, IL-10, IFN-γ (Tg) IL-4 (Tg)</td>
<td>Cytokine production abnormalities</td>
</tr>
<tr>
<td>ERα</td>
<td>Defective hormone signaling</td>
</tr>
</tbody>
</table>

No single gene defect is sufficient for lupus development
In the lupus prone NZM mouse, *Sle1*+*2*+*3* are the major susceptibility loci. Triple congenic mice on a B6 background reconstitute the pathology (Koch’s postulate).

*Sle1*  
C’, Sap  
Loss of tolerance to chromatin leading to ANA production, B cell defect

*Sle2*  
Ipr, yaa, Blys  
Progressive expansion of autoimmune response with epitope spreading

*Sle3*  
Dysregulation of CD4+ T cells, non T-cell autonomous

*Sle6*  
FcR  
End organ damage culminating in fatal lupus
Physiopathologie du lupus

D’après Morel et al, Immunity 1999

Sle1  Sle2,3  Sle6
Lpr, gld, yaa

NZM loci

Rupture de tolérance / chromatine ANA
Expansion, ANA ++
Lésion d’organe Lupus fatal
Sle1, a cluster of functionally related genes

- Human and mouse studies converge on mouse telomeric chr1 and human 1q21-44
- Mutations of genes encoded in this region FcgRIIA, FcgRIIIA, FcRg and SAP modulate lupus susceptibility
- Backcross studies B6.Sle1
  - selective loss of tolerance to chromatin (H2A/H2B/DNA subnucleosomes) and spontaneously activated T and B lymphocytes
  - But normal immune responses and normal lymphocyte apoptosis
Contribution of B cells in Sle1 effect

• Bone marrow reconstitution studies:
  – B6.Sle1 bm -> B6: autoantibodies, activ CD4+
  – B6 bm -> B6.Sle1: normal

• Cotransfer of bone marrows: B6’, B6.Sle1
  – Ig Allotype marker: « a » for B6’, « b » for B6.Sle1
  – Autoantibodies of the « b » allotype Activ B cells of the « b » allotype

• B cells carry the Sle1 phenotype in a cell-autonomous fashion but autoantibodies are non nephrogenic
Dissecting further Sle1

- Genetic recombinants in Sle1 -> 1a, 1b, 1c using new microsatellite markers polymorphic between B6 and B6.Sle1
- Congenic lines on B6 background (> 800 mice analysed !!)
- Autoantibodies and activ CD4+ cells:
  - 1b > 1a and 1c
  - 1a + 1b = 1a + 1b + 1c = WT Sle1
- Nephrogenic Ab ? : cross Sle1 congenic mice on the NZW background and analyse proliferative GN: GN correlates with Sle1b in (NZWxB6.Sle1)F1 and activ CD4+ T cells but not with higher autoAb titers
- Additional locus in Sle1b controls the extent of GN (Sle1d => Suppressive effect ?)
- Candidate gene for Sle1c: CR2 ?
Regulating the Sle1 effect

Sle1

Sle2,3
Lpr, gld, yaa

Sle6

Rupture de tolérance / chromatine
ANA

Expansion,
ANA ++

Lésion d’organe
Lupus fatal

Sles1 is present in the lupus-prone NZM strain and attenuates the pathology in this strain via epistatic interactions.
Physiopathology of Lupus

Apoptosis defect

Defective clearance Of apoptotic cells

Complement deficiency

Loss of tolerance To apoptotic self

Hyperactivation of self reactive B cells

Immune complex deposition

The clearance hypothesis

The tolerance hypothesis
From defective clearance
to loss of tolerance
Apoptotic cells must be cleared

At least two steps:
- clearing
- digesting and masking
Early exposure of ‘eat me’ flags

Waste disposal
- Removal of cell corpses
- Prevention of leakage of contents from dying cells

Degradation and processing

Presentation of peptides MHC

Altered secretion

New meaning
- Suppression of inflammation (TGF-β1↑, PGE₂↑, TNF-α↓)
- Modulation of cell killing (NO↓, CD95L↑)
- Regulation of immune response (via class I and II MHC)
Phagocyte receptors
Corps apoptotique

« Eat me » signals

Fonctions différentes des récepteurs
Tether -> Trigger

Macrophage

Distribution tissulaire des récepteurs
- différentielle
- régulée

CHO
iC3b
PS
C1q
b2GPI
TSP
ox LDL

CD14
lectin
b2 intégrine

PS-R
C1q-R
CR1
CD36, intégrines
CD68 ...

« Cool down » or
« Turn me on » signals
Phenotypes of SLE patients

- Low CRP levels in serum
- Reduced DNAse 1 activity
- C’ defects
- Reduction in the degradation capacity of necrotic-derived chromatin
Déficit en complément et prédisposition au lupus

% LUPUS

<table>
<thead>
<tr>
<th></th>
<th>Homme</th>
<th>Concordance entre jumeaux</th>
<th>Souris</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>93</td>
<td>90</td>
<td>++</td>
</tr>
<tr>
<td>C1r/s</td>
<td>57</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>C4A/B</td>
<td>75</td>
<td>80</td>
<td>++</td>
</tr>
<tr>
<td>C2</td>
<td>10</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>rare</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MAC</td>
<td>rare</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Expérience:

1. Péritonite stérile (thioglycolate IP) -> macrophages péritonéaux
2. Injection de thymocytes apoptotiques -> clairance in vivo ?

<table>
<thead>
<tr>
<th>Souris</th>
<th>Clairance</th>
<th>Lupus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contrôle</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>C1q KO</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>C4 KO</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>C3 KO</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusion:

La déplétion en C1q entrave la phagocytose des corps apoptotiques et prédispose au lupus
Cellules apoptotiques, source d’autoantigènes ?

**Auto-anticorps du lupus**

a) Ag intracellulaires  
   -> chromatine intracellulaire  
   -> spliceosome  
   -> snRNP

b) Antigènes membranaires : Phospholipides (PS)

c) Protéines plasmatiques : β2 glycoprot I, C1q

Ag ubiquitaires, abondants ⇒ rupture de tolérance improbable

**Corps apoptotiques**

<table>
<thead>
<tr>
<th>a) Ag intracellulaires</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>b) Antigènes membranaires</td>
<td>+</td>
</tr>
<tr>
<td>c) Protéines plasmatiques</td>
<td>+</td>
</tr>
</tbody>
</table>

NéoAg ?  
Externalisation Ag  
CA in vivo rares ?
Opsonisation via C1q

Flexibility and versatility of ligand recognition

Heterotrimer
Modular assembly
Charge
Spatial orientation

Important role in
-Clearance
  - Altered self
  - Infectious nonself
-Inflammation

Symmetrical trimer of β-sandwich subunits (TNF superfamily)
C1q-based recognition

• C1q engagement leading to
  – MAC activation
    • immune complexes (IgG) -> MAC
    • non immune molecules (polyanions such as LPS, DNA, RNA, polysaccharides, viral membranes, Prp, and unidentified microbial components)
  – opsonisation
    • CRP, SAP, pentraxin : regulators of opsonin activity towards altered self debris
    • Fibronectin, fibrin, … : involved in wound healing
    • natural antibodies

• In conclusion:
  – Highly diversified recognition modules with combinatorial potential
  – Recognition of ALTERED SELF versus NON-SELF recognition leads to differential effector functions (opsonisation versus lysis ?)
Interaction of CRP with C1q

Recognition face binds ligand: « masking effect »?
Effector face binds C1q or FcγR
Effector function: opsonin?

• Activator of C’–mediated opsonisation (up to C3 convertase) due to recruitment or induction of inhibitory factors (H, CD59, DAF, …)

• FcR-mediated phagocytosis
CRP role

• Hiding SELF?

• rather anti-inflammatory towards self components (ex: lupus) ->

• proinflammatory towards bacteria, provides protection towards PC and PE+ bacteria
Clairance des IC: rôle du C’ et des GR

D’après Immunological Rev, 2001
C’ et inflammation

C’ consommé en phase active de lupus
  - C1q, C2, C4, plus rarement C3 (formes sévères)

Dépôt de C’  -> associé aux IC
  -> pas strictement corrélé aux lésions tissulaires

Clairance des IC : CR versus FcR ?

Rôle des anticorps anti-C1q ?
  -> Vascularite urticariante (HUVS)
  -> Lupus (1/3 patients +)
Le rôle des auto-anticorps ?

1. Expression des autoAg à la membrane des corps apoptotiques
2. Clairance médiée par les auto Ac -> capture FcγR-dépendante
3. Consommation de C1q -> perte du pouvoir « solubilisant » du C’ pour les complexes immuns
4. Conséquence systémique -> dépôts d’IC dans les tissus
5. Début des lésions tissulaires
Corps apoptotique

C’PCR

Macrophage

CR1, CD36, …. FcR

Cellule dendritique

TGFβ, IL-10

IL-1, TNFα

C’PCR

Auto Ac

Corps apoptotique

Tolérance

Rupture de tolérance

Présentation croisée

FcR ?
Autoantibody-mediated pathogenesis of SLE.

Apoptosis defect

IC formation

Lymphocyte activation in GC

Tissue inflammation and lesions

But not all autoAb and IC are pathogenic
Impact on B cell function?

- Excessive autoantigen production
- Engagement of immunogenic rather than tolerogenic Ag presentation
- Hyperactivation of B cells
- Abnormal regulation of Ig switch and production of high affinity autoantibodies
- Abnormal capture / clearance of IC complexes
- Abnormal immune deposits, innate cell activation and tissue response to inflammation
Pathogenic role for B cells

- Efficacy of B cell depletion in some AID
  - Rituximab: anti-CD20 Ab
  - CD20 marker of B cells, absent from plasma cells
  - Modes of action
    - B cell -> autoAb depletion
    - Delayed reconstitution
    - Abrogation of B-cell dependent downstream effectors including T-B cooperation (i.e. improvement of lupus by anti-CD40L mAb) and neolymphogenesis

- ITP: no direct correlation between auto-Ab levels and B cell depletion using Rituximab

- RA: impact on rhumatoid factor levels
Modulation of B cell survival

- The BLys/BAFF pathway, members of the TNF/TNF-R family
- Required for B cell survival
- Increased serum BLys/BAFF levels in Sjogren, SLE patients
- Transgenic BLys mice develop Sjogren’s and lupus-like diseases
- Blocking BLys signals improves SLE in mouse
The case of Autoantibodies

• Directly pathogenic (myasthenia (AChR), FVIII deficiencies, Graves’ disease (TSH), autoimmune hemolytic anemia, neutropenia, thrombocytopenia)

• FcR dependent effects and innate cell degranulation

• Immune complexes (mixed cryoglobulinemia, SLE, RA, vasculitis)
Immune complexes, C’ and FcR

• IC deposition, C’ activation
  – Kidney, lung, blood vessels -> kidney failure, lung hemorrhages, vasculitis
  – Hepatitis C associated cryoglobulinemia (purpura, glomerulonephritis, motoneuritis)
  – Role of C’: anti-C5 inhibitor antibody (eculizumab) in RA

• IC-mediated FcR activation
  – Linked polymorphism of low affinity IgG FcR with AID (CD32, CD16) -> impact on phagocytic property of APC
  – IVIG useful in idiopathic thombopenic purpura (engagement of inhibitory FcγRIIB)
Immune complexes

Polyclonal Ab
Multi-epitope Ag binding
Optimal concentrations

Deposition on basal membranes
and inflammation/destruction
Lupus nephritis: pathogenic Ab

Break of tolerance → Autoantibody production

Elevated serum anti-dsDNA levels → Immune complex (IC) accumulation

Genes affecting:
- Autoantibody specificity
- IC localization
- Inflammatory response to IC
- Intrinsic kidney susceptibility to end-organ damage

GN → Glomerular damage
Proteinuria
Loss of kidney function

Current Opinion in Immunology
Effector mechanisms for Ab
# Lymphocyte activation defect

<table>
<thead>
<tr>
<th>Gene</th>
<th>OMIM(^a)</th>
<th>Cytogenetic location</th>
<th>Associated allele(s)</th>
</tr>
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<tbody>
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<td>IL10</td>
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<td>1q31-32</td>
<td>Multiple alleles</td>
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<td>Multiple alleles</td>
</tr>
</tbody>
</table>

\(^a\)Online Mendelian Inheritance in Man (OMIM)
Gadd45a deficiency

- Growth-arrest and DNA damage-inducible gene
- Downstream effector of p53
- Participates to growth arrest, genomic stability and apoptosis
- KO mouse: lupus-like disease
Excessive inflammation
Lupus: IFN$\alpha$-driven disease?

Chronic IFN pathway activation

Virus?
The IFN signature
Abnormal lymphoid organs
Abnormal cell positioning?