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 ?)
### Genetic predisposition to AIDs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Concordance rate (%)</th>
<th>Population prevalence</th>
<th>Genetic influence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monozygotic twins</td>
<td>Dizygotic twins</td>
<td>Non-twin siblings</td>
</tr>
<tr>
<td>Diabetes</td>
<td>30-50</td>
<td>0-13</td>
<td>6</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>25</td>
<td>0-5</td>
<td>3-5</td>
</tr>
<tr>
<td>Lupus</td>
<td>24-57</td>
<td>2-5</td>
<td>2-5</td>
</tr>
<tr>
<td>Rhumatoid arthritis</td>
<td>12-15</td>
<td>3-4</td>
<td>2-4</td>
</tr>
</tbody>
</table>

- Multigenic
- Genetic influence
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
MHC: the most potent susceptibility locus

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

Other genes

MHC identical siblings

MHC weight

15%
# Gene clusters

<table>
<thead>
<tr>
<th>Cluster type</th>
<th>Total number of loci</th>
<th>Number of protein-coding loci</th>
<th>Number of pseudogene/transcript loci</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene superclusters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histone</td>
<td>66</td>
<td>55</td>
<td>11</td>
</tr>
<tr>
<td>HLA class I</td>
<td>26</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>tRNA</td>
<td>157</td>
<td>151</td>
<td>8</td>
</tr>
<tr>
<td>Butyrophilin</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Olfactory receptor*</td>
<td>34</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Zinc finger protein</td>
<td>36</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td><strong>Gene clusters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solite carrier 17A</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Vomeronasal receptor</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Tumour necrosis factor</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lymphocyte antigen-6</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Heat shock protein</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>HLA class II*</td>
<td>24</td>
<td>15</td>
<td>9</td>
</tr>
</tbody>
</table>

*The distribution of olfactory loci between the gene and pseudogene categories is dependent on haplotype. *The number of loci in the HLA class II supercluster varies between different haplotypes.

Please see text for details on each individual cluster. xMHC, extended major histocompatibility complex.
Association with most autoimmune diseases

<table>
<thead>
<tr>
<th>Category</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen processing/presentation</td>
<td>HLA-A, -B, -C, -DMA, -DMB, -DOA, -DOB, -DPA1, -DPB1, -DQA1, -DQA2, -DQB1, -DQB2, -DRA, -DRB1, -DRB3, -DRB4, -DRB5, PRSS16, PSMB8, PSMB9, TAP1, TAP2, TAPBP; UBD</td>
</tr>
<tr>
<td>Immunoglobulin superfamily</td>
<td>AGER; BTN1A1, BTN2A1, BTN2A2, BTN2A3, BTN3A1, BTN3A2, BTN3A3, BTN1L2; C6orf25; MOG</td>
</tr>
<tr>
<td>Inflammation</td>
<td>ABCF1; AIF1; DAXX; IER3; LST1; LTA, LTB; NCR3; TNF</td>
</tr>
<tr>
<td>Complement cascade</td>
<td>BF; C2, C4A, C4B</td>
</tr>
<tr>
<td>Non-classical MHC class I</td>
<td>HLA-E, HLA-F, HLA-G; HFE</td>
</tr>
<tr>
<td>Immune regulation</td>
<td>NFKBIL1, RXRB, FKBPL</td>
</tr>
<tr>
<td>Stress response</td>
<td>HSPA1A, HSPA1B, HSPA1L; MICA, MICB</td>
</tr>
<tr>
<td>Gene symbol</td>
<td>Relationship to disease*</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>xMHC extended class I region</strong></td>
<td></td>
</tr>
<tr>
<td>HFE</td>
<td>Causes haemochromatosis; associated with arthropathy, multiple sclerosis, hepatocellular carcinoma</td>
</tr>
<tr>
<td>UBD</td>
<td>Associated with gastrointestinal and gynaecological malignancies</td>
</tr>
<tr>
<td>MOG</td>
<td>Associated with multiple sclerosis</td>
</tr>
<tr>
<td><strong>xMHC class I region</strong></td>
<td></td>
</tr>
<tr>
<td>HLA-G</td>
<td>Associated with <em>Pemphigus vulgaris</em> in Jewish patients</td>
</tr>
<tr>
<td>HLA-A</td>
<td>Associated with autoimmune diseases; for example, birdshot chorioretinopathy</td>
</tr>
<tr>
<td>HLA-E</td>
<td>Associated with type 1 <em>Diabetes mellitus</em>; also influences age of onset of disease</td>
</tr>
<tr>
<td>MDC1</td>
<td>Associated with inadequate DNA damage responses owing to MDC1-deficiency</td>
</tr>
<tr>
<td>CDSN</td>
<td>Causes hypotrichosis simplex of the scalp</td>
</tr>
<tr>
<td>PSORS1C1</td>
<td>Associated with psoriasis</td>
</tr>
<tr>
<td>PSORS1C2</td>
<td>Associated with psoriasis</td>
</tr>
<tr>
<td>C6orf18</td>
<td>Associated with psoriasis</td>
</tr>
<tr>
<td>HLA-C</td>
<td>Associated with autoimmune diseases; for example, psoriasis</td>
</tr>
<tr>
<td>HLA-B</td>
<td>Associated with autoimmune diseases; for example, ankylosing spondylitis or Behcet disease</td>
</tr>
<tr>
<td>MICA</td>
<td>Associated with autoimmune diseases; for example, rheumatoid arthritis and coeliac disease</td>
</tr>
<tr>
<td>MICB</td>
<td>Associated with coeliac disease</td>
</tr>
<tr>
<td><strong>xMHC class III region</strong></td>
<td></td>
</tr>
<tr>
<td>NFkBII1</td>
<td>Associated with rheumatoid arthritis</td>
</tr>
<tr>
<td>LTA</td>
<td>Associated with myocardial infarction</td>
</tr>
<tr>
<td>TNF</td>
<td>Associated with septic shock, cerebral malaria</td>
</tr>
<tr>
<td>LTB</td>
<td>Associated with infective/inflammatory diseases</td>
</tr>
<tr>
<td>NCR3</td>
<td>Associated with impairment of NK cell function in HIV-1 infected patients</td>
</tr>
</tbody>
</table>
### xMHC class III region

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFKBIL1</td>
<td>Associated with rheumatoid arthritis</td>
</tr>
<tr>
<td>LTA</td>
<td>Associated with myocardial infarction</td>
</tr>
<tr>
<td>TNF</td>
<td>Associated with septic shock, cerebral malaria</td>
</tr>
<tr>
<td>LTB</td>
<td>Associated with infective/inflammatory diseases</td>
</tr>
<tr>
<td>NCR3</td>
<td>Associated with impairment of NK cell function in HIV-1 infected patients</td>
</tr>
<tr>
<td>BAT2</td>
<td>Associated with influence on age at onset of type 1 Diabetes mellitus</td>
</tr>
<tr>
<td>NEU1</td>
<td>Causes type I and II sialidosis</td>
</tr>
<tr>
<td>C2</td>
<td>Causes C2 deficiency</td>
</tr>
<tr>
<td>C4B</td>
<td>Causes C4 deficiency</td>
</tr>
<tr>
<td>C4A</td>
<td>Causes C4 deficiency</td>
</tr>
<tr>
<td>CYP21A2</td>
<td>Causes several disorders owing to 21-hydroxylase deficiency</td>
</tr>
<tr>
<td>TNXB</td>
<td>Causes Ehlers–Danlos syndrome (hypermobility type) owing to tenascin X deficiency</td>
</tr>
<tr>
<td>AGER</td>
<td>Associated with amplification of inflammatory responses in rheumatoid arthritis</td>
</tr>
</tbody>
</table>

### xMHC class II region

<table>
<thead>
<tr>
<th>Loci</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR</td>
<td>Associated with autoimmune diseases; for example, rheumatoid arthritis, type 1 and type 2 Diabetes mellitus</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>Associated with autoimmune diseases; for example, narcolepsy</td>
</tr>
<tr>
<td>TAP2</td>
<td>Causes bare lymphocyte syndrome type 1 owing to TAP2-deficiency; associated with various diseases; for example, rheumatoid arthritis</td>
</tr>
<tr>
<td>TAP1</td>
<td>Causes bare lymphocyte syndrome type 1 owing to TAP1-deficiency; associated with various diseases; for example, vitiligo in Caucasian patients that are young in age at onset</td>
</tr>
<tr>
<td>BRD2</td>
<td>Associated with juvenile myoclonic epilepsy</td>
</tr>
<tr>
<td>HLA-DP</td>
<td>Associated with autoimmune diseases; for example, chronic beryllium disease</td>
</tr>
</tbody>
</table>

### xMHC extended class II region

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL11A2</td>
<td>Causes autosomal dominant deafness (DFNA13) and several other diseases</td>
</tr>
<tr>
<td>TAPBP</td>
<td>Causes bare lymphocyte syndrome type 1 owing to TAPBP-deficiency</td>
</tr>
</tbody>
</table>
Difficulty of linkage analysis

• Most MHC-linked diseases are multigenic

• Strong linkage disequilibrium in the MHC locus
# Conserved Extended Haplotypes

<table>
<thead>
<tr>
<th>CEH type</th>
<th>CEHs ( [\text{HLA-B, complotype, DR}] )</th>
<th>Ratio (T1D:normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptibility</td>
<td>B62, SC31, DR4</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>B18, F1C30, DR3</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>
</tr>
<tr>
<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>
</tr>
<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>

T1D And MHC Conserved Extended Haplotypes
MHC susceptibility locus for T1D

- HLA-DQβ1, HLA-DRβ1: still candidate alleles, not definitively proven
- Mouse equivalent: I-A^g7 (Tg allo-MHC protective)
- Frequency of MHC susceptibility alleles in Caucasian population: 53% => More frequent than protective alleles!!
- Inheritance
  - Recessive mode for susceptibility
  - Dominant mode for protection
- Incomplete penetrance of susceptibility alleles
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
MHC peptide binding groove
Penetrance of susceptibility genes

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

- Environmental triggers (not proven !)

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

- Importance of other non-MHC genetic loci
Genetic heterogeneity

- Association studies differ between ethnic groups
- Genetic localisations of AIDs vary between disease models
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
Genomic Regions:

Breaks in LD occur once every 200kB in the genome

Two types of regions can be defined
- strong linkage disequilibrium -> tag-SNPs (70-80 % genome)
- low linkage disequilibrium (20% genome)
  -> no hope of detecting rare susceptibility variants
Towards a complete SNP map

- HapMap project: non contiguous resequencing of 5-60 different chromosomes
- End of 2005: coverage 1 SNP per 5kB
- Hope: use 75000 tag SNPs to cover 25% of the human genome
Disease susceptibility variants
Minor Allele frequency / phenotypic effect

Effect size of the SNP

Sample size required (number of individuals)
Frequency of disease-susceptibility allele

Nature Reviews Genetics
Allele frequencies for susceptibility loci: two extreme views

- **CDCV hypothesis**: Common diseases are the results of common variants

- **Disease heterogeneity hypothesis**: Disease susceptibility is due to distinct genetic variants in different individuals and disease susceptibility alleles have low population frequencies (MAF < 0.01)
Requires Epistatic Interactions Between Several loci
SNPs leading to non synonymous nucleotide changes -> protein variant

SNPs under positive selection:
- anti-infectious response -> autoimmune diseases
- adaptation to starvation -> type 2 diabetes
SNPs and diseases

Estimate: hundreds of common and rare variants contributing to the familial clustering of each common human disease
Large number of variants with small effects

• Rather than affecting protein structure, mutations affect
  – Expression levels (polymorphism in non-coding regulatory sequences)
  – Splice variants
• Little familial recurrence risk (for odd ratio 1.1-1.5)
Epistasis: how to score gene interactions in complex genetic diseases?

- Bateson’s definition: one gene cancelling out the effet of another
- Rare situation, rather moderate effects only detected in defined genetic contexts
- Pitfalls: hidden population stratification and admixture
- Need for subgroup analysis in well defined cohorts
Few success stories in linkage analyses

• Inflammatory bowel disease:
  – NOD2 gene, intracellular bacterial stress sensor, 2-fold impact on disease risk!
  – DLG5 gene, maintenance of epithelial integrity, 1.49 risk ratio

• Schizophrenia:
  – Neuregulin gene regulator of NMDA signalling (glutamate), synaptic plasticity, 1.8 risk ratio

• Type 1 diabetes:
  – CTLA4, negative regulator of T cell activation,
Type 1 diabetes:
a need for refined phenotypic analyses

Human or rodent models:
  NOD mouse
  BB rat
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
CAUSES OF T1D

A combined effect of genetic and environmental factors lead to different forms of the same disease
Genetic predisposition

- Twin studies: concordance rate around 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
Human IDDM loci
MOUSE Idd loci
Environmental versus genetic factors

- Genetically identical human twins: around 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
  - 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)
Virus can be a trigger

Expression transgénique d’un antigène du LCMV sous le contrôle d’un promoteur insuline

Diabète

Ignorance = Tolérance

Initiation

Infection

Pas de virus détectable

Naissance

LCMV

TEMPS
### Comparison of autoimmune diabetes in NOD mice and humans

<table>
<thead>
<tr>
<th>Similarities</th>
<th>Humans</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic predisposition and polygenic trait</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MHC-loci contribution</td>
<td>Multiple</td>
<td>Multiple</td>
</tr>
<tr>
<td>Environmental Influence</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Defective peripheral immune regulation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Impaired dendritic-cell maturation and function</td>
<td>Possibly</td>
<td>Yes</td>
</tr>
<tr>
<td>Disease transmission with bone-marrow transplantation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Autoantigens</td>
<td>GAD65, IA2, insulin and 38 kD</td>
<td>GAD65, IA2, insulin and 38 kD</td>
</tr>
<tr>
<td>Initiating autoantigen</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Delayed onset with immunosuppression</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Islet autoimmunity linked to early gluten exposure</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Differences

| Endogenous retrovirus in β-cells                | Unclear      | Yes*     |
| T-cell-driven insulitis                         | Mild         | Severe   |
| Humoral reactivity to β-cells                   | GAD65, IA2 and insulin | Insulin |
| Insulin gene                                    | One          | Two      |
| GAD65 expression by β-cells                    | Yes          | No       |
| Incidence                                       | 0.25–0.40%   | > 80%    |
| Incidence in genetically susceptible subjects   | 5–30%        | > 80%    |

### Mechanisms

| Susceptibility of β-cells to STZ or NO in vitro* | Only at high concentrations | Very susceptible |
| Maternal autoantibodies                          | Potentially reduced risk of T1D | Diabetogenic |
| B cells required                                 | No            | Yes      |
| Successful intervention therapies                | Pending       | > 195    |
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)
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
Autoimmunity in type 1 diabetes

Autoantigen release → Priming → Autoimmune lymphoid repertoire → Recruitment → Expansion → Differentiation → Regulation → Loss of tolerance → Diabes

Islet beta cells

TIME
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
Genetic approaches

- Linkage analysis in families i.e. cosegregation of marker and disease locus alleles (not necessarily close)

- Association analysis in populations i.e. close physical linkage between marker and disease locus alleles (linkage disequilibrium)

- Positional candidate mapping

- Probing gene interactions (epistasis) -> allelic associations

- Combining genetic (recombinant mouse strains) and phenotypic approaches (sensitized phenotype)
The congenic mouse approach

- Refine intervals of susceptibility by backcrossing resistance loci on susceptible genetic background

-> narrow down to 0.5-3 Mb !

- Determine gene content, cross with expression and functional data

- Identify sequence polymorphism (SNPs)

- Match with precise phenotypes
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^g7 but not in NOD mice

• The B6 phenotype is associated with early insulitis

• Backcross on B6^g7 shows rapid loss of diabetes penetrance suggesting that few gene loci contribute to the B6 phenotype
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*
Structural features of diabetogenic MHC

- Similarities between crystal structures of DQ8 and I-A\textsuperscript{\textgamma}
- Binding of similar sets of peptides with an acidic charge
- Importance of the P9 pocket carrying a positive charge due to the lack of an Asp residue in position 57 of the beta chain
- DQ8 and not DQ6 transgenic mice coexpressing B7-1 on islet cells show increased diabetes incidence on the B6 background
MHC contribution to peptide recognition

- NOD I-A\textsuperscript{b} or B6 H-2\textsuperscript{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?
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
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\(^{g7}\) 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.
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
- Insulin gene expression is promiscuous and depends on the AIRE transcription factor in thymus
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.
Tolérance centrale ou périphérique ?

Expression du transgène

Lymphocyte T périphérique

Diabète

ignorant
tolérant
délétè

rapide

progressif

absent
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
Phenotypic approaches
Multiple immunological defects in type 1 diabetes

- Autoantigen expression
  - GAD, insulin, IA, ...

- HLA
  - Priming

- Autoimmune lymphoid repertoire

- STRESS

- Recruitment

- Chemokines

- Expansion

- Differentiation

- Regulation

- CD4+, Th1 > Th2
  - Regulatory T/NKT?

- CD8+, B, CPA
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)
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
Both CD4+ and CD8+ are required

• Transfer into SCID NOD with CD4+ and CD8+ lymphocytes

• Inhibition by addition of Treg

• Transfer using T cell clones with a single antigenic specificity
Control of lymphoid effectors
Cytokines, signalling modules

Ag selection  Expansion  Differentiation into effectors  Selection by apoptosis  Differentiation into memory cells

Fréquency 1/10^5-10^6  IL-2  IL-4  IL-12  IFNg  TGFb  Fas/Fas-L  CTLA-4  IL-15, IL-7
Th1 / Th2 / Th3 balance

- Th1: IL-12, IFNγ
- Th2: IL-4
- Th3: IL-2, IL-10, TGFβ

- Pro-inflammatory
- Auxiliary for B's Switch
- Régulatory
- Dominant effect
Th1 > Th2 in T1D

- 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
Defective 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 T reg 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 T reg numbers and protects from streptozotocine induced diabetes

- Modulating islet cell apoptosis
Coopération DC-T

SIGNAL 1: CAPTURE ET PRESENTATION DE L’ANTIGÈNE

+ SIGNAL 2 : DANGER ET COSTIMULATION

EXPANSION CLONALE, INFLAMMATION,
et PRODUCTION D’EFFETTEURS CD4+ et CD8+
# Diabetes incidence in mutant NOD mice

<table>
<thead>
<tr>
<th>Protein</th>
<th>Change</th>
<th>Effect</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>
</tr>
</tbody>
</table>
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
Cbl-b, gate-keeper de l’activation T

KO Cbl-b : affranchissement du besoin en cosignal
=> AUTOIMMUNITE
CTLA4
Human 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
Tolérance = contrôle du cosignal

Cellule dendritique immunogène : fournit le cosignal

Déficit IL-10 Pathologie intestinale inflammatoire
Déficit CTLA-4 Lymphoprolifération et Autoimmunité
Human CTLA4 variant

Expression of the human CTLA-4 mRNA isoforms correlates with genotype
CTLA4
Mouse *Idd5.1*

B6/NOD SNP in ligand-binding exon 2
-> modifies exonic splicing silencer motif
-> novel CTLA4 isoform
-> genetic control: high expression in B10 versus NOD allele
-> functional validation: knock in in NOD background -> confers resistance?
Diabetes: *Idd* complementarity

**Idd5** chr 1

- **Idd5.1** 1.5 cM, 45 genes
  - CTLA4, negative regulator of T cell activation
  - ICOS, costimulator of T cell

- **Idd5.2** 5.1 cM, 4 genes
  - Nramp1 Cation transporter
  - Resistance to intracellular pathogens

Partial protection 30% D 7mo

**Idd3** chr 3

Combined with Idd5
- > full protection
Frequency of diabetes in female *Idd5* congeneric mice
The *Idd5.1* region controls expression levels of liCTLA-4 in congenic strains.
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?
**Effect of cytokines on diabetes incidence**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Effect</th>
<th>Function</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></td>
<td>late abolished</td>
<td>Death of autoreactive cells</td>
</tr>
</tbody>
</table>
Lymphocytes B
Diversification antigénique

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
Epitopes cryptiques

HIV

Infection et réplication dans lymphocytes activés

Augmente la production endogène de vinculine

Cytopathique

Complexes peptides vinculine / CMH
-> CPA

CTL CD8 anti-vinculine

Auto-immunisation vis à vis d’une protéine ubiquitaire
NKT cells

- Reduced numbers in NOD mice
- Injection of NKT cells protects from diabetes
- Activating NKT cell ligands block diabetes ($\alpha$Gal 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
Apoptose et inflammation

Elimination discrète !

Agitateurs
Toll, IFN, TNF, IL-1

Convocation du lymphocyte et identification du cadavre

Cross-présentation
Co-stimulation
Recrutement
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.
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
<table>
<thead>
<tr>
<th>Transgenic mice</th>
<th>TCR</th>
<th>Gene(s)</th>
<th>Outcome</th>
<th>Clinical equivalent/relevance</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BDC2.5 x NOD</td>
<td>In some cases, acceleration of T1D occurs. No spontaneous diabetes occurs in non-NOD mice except in neonatal TCR-transgenic mice expressing HA under the RIP. Peripheral, non-destructive insulins occur.</td>
<td>Presence of T cells with specificity for islet antigens does not always lead to breaking of tolerance, which requires a strong trigger and/or correct genetic background, as provided in NOD mice. Non-destructive autoimmune insulins does not commonly occur in humans.</td>
<td>4.2, 22, 53, 57, 60</td>
</tr>
<tr>
<td>MHC</td>
<td>HLA-DR</td>
<td>No spontaneous T1D when overexpressed by β-cells. unless β-cells are overloaded with neo-proteins. Humanized mice can be important models.</td>
<td>MHC class II contributes to T1D but does not lead to breaking of tolerance. Suitable for studying human MHC-class II-restricted T cells and MHC-associated genetic protection.</td>
<td>63, 116, 117</td>
<td></td>
</tr>
<tr>
<td>Necanitogen</td>
<td>HA/HEL</td>
<td>Non-tolerant antigens alone do not lead to autoimmunity. T cells need to be activated in sufficient numbers to lead to sufficient β-cell destruction.</td>
<td>Necanitogens alone do not lead to autoimmunity; inflammation is required. In RIP-LCMV transgenic mice, viral infection constitutes a sufficiently strong stimulus for T1D development. Similarly, cross-presentation can be crucial for propagating the autoimmune process.</td>
<td>51, 53, 65</td>
<td></td>
</tr>
<tr>
<td>Co-stimulation</td>
<td>Cd80/Cd86</td>
<td>In some cases, spontaneous diabetes can occur when these molecules are expressed under the RIP. T1D development is markedly accelerated.</td>
<td>Local clonal expansion of lymphocytes in islets promotes T1D. If β-cells function as professional APCs, T1D development is accelerated.</td>
<td>118, 119</td>
<td></td>
</tr>
<tr>
<td>Cytokine</td>
<td>Il-4, Il-7, Tnf</td>
<td>All of these accelerate T1D development. Il-4, Il-7 or Tnf plus Cd80 overexpression by β-cells can lead to spontaneous diabetes without other stimuli, together with presentation of autoantigens.</td>
<td>Inflammatory cytokines such as these are expected to accelerate development of human T1D. Contribution of virus could be explained by this.</td>
<td>56, 61, 62, 65, 88, 78, 80, 81, 88</td>
<td></td>
</tr>
<tr>
<td>Anti-apoptosis</td>
<td>Fas, Bcl-2</td>
<td>No marked prevention of T1D in most models.</td>
<td>Redundant mechanisms of β-cell destruction exist.</td>
<td>26, 75, 120, 121</td>
<td></td>
</tr>
<tr>
<td>Autoantigen</td>
<td>Gad65</td>
<td>GAD65 expression in the thymus and the ensuing tolerance does not prevent T1D. Insulin expression in the thymus causes strong reduction of T1D incidence.</td>
<td>There is more than one autoantigen that can drive the autoimmune process, showing the importance of redundancy. Insulin is a strong primary candidate autoantigen.</td>
<td>54, 66, 67, 110</td>
<td></td>
</tr>
</tbody>
</table>

**Knockout mice**

<p>| Autoantigen | Gad65 | GAD65 expression in the thymus and the ensuing tolerance does not prevent T1D. Insulin expression in the thymus causes strong reduction of T1D incidence. | Different autoantigens have different roles in the autoimmune process. Similar to many of the known autoantigens, GAD might be non-essential for T1D. Mouse β-cells do not express GAD65; human β-cells do. Humans do not have the gene encoding insulin-2. | 55, 66, 67, 75 |
| Cytokine | Il-4, Il-10, Tnf/β, Il-17 | Acceleration of T1D in most, but not all, cases. | Regulatory cytokines that dampen T1D exist and can be used therapeutically. | 58, 78, 79, 122, 123 |</p>
<table>
<thead>
<tr>
<th>Observation</th>
<th>Appropriate action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early prevention of disease (for example, at 4 weeks of age) in NOD mice is</td>
<td>Focus attempts at early intervention on agents that are suitable for such use in humans (with respect to ethics, safety and cost)</td>
</tr>
<tr>
<td>easy</td>
<td></td>
</tr>
<tr>
<td>Non-specific pathogens influence the disease rate in NOD mice</td>
<td>Carry out investigations in specific-pathogen-free environments</td>
</tr>
<tr>
<td>Late interventions for disease prevention or reversal in NOD mice are</td>
<td>Attempt more studies of agents at the onset of disease, with the goal of disease reversal or retention of C-peptide function</td>
</tr>
<tr>
<td>difficult</td>
<td></td>
</tr>
<tr>
<td>Not all disease interventions are safe (for example, they can induce shock)</td>
<td>Test dosage and toxicity</td>
</tr>
<tr>
<td>Many reports use the word ‘prevent’ but ‘delay’ might be more appropriate</td>
<td>Establish criteria that define ‘marginal delay’, ‘significant delay’ and ‘absolute prevention’</td>
</tr>
<tr>
<td>Most studies of type 1 diabetes in animal models use NOD mice, a practice</td>
<td>Attempt prevention-based interventions using other animal models (that is, rats and/or other mouse models)</td>
</tr>
<tr>
<td>that carries risks for application to type 1 diabetes in humans</td>
<td></td>
</tr>
<tr>
<td>Animal models are not chosen according to the questions asked but based on</td>
<td>Re-evaluate the relevance of each model to the questions asked. Avoid making animal models ‘gold standards’ for all questions asked</td>
</tr>
<tr>
<td>their frequency of use in the scientific community</td>
<td></td>
</tr>
<tr>
<td>Animal models are used to implicate genetic defects (using gene ‘knockouts’)</td>
<td>Avoid this type of ‘chicken or egg’ loop and validate immune defects first in humans, or report the finding made in the animal model while citing the</td>
</tr>
<tr>
<td>in the immunopathogenesis of human type 1 diabetes, although humans do not</td>
<td>appropriate caveats for translating this data to apply to humans. Gene-knockout models might be useful tools to dissect immunological pathways, yet few</td>
</tr>
<tr>
<td>necessarily show the defect(s) that causes diabetes in the animal model</td>
<td>human diseases are based on a single gene defect</td>
</tr>
</tbody>
</table>

NOD, non-obese diabetic.
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