Immunogenetic surveillance of the HIV/AIDS pandemic

Henry Stephens

UCL Centre for Nephrology & Anthony Nolan Laboratories
What is Immunogenetics?

• Identification of human leukocyte antigen (HLA) proteins (phenotype), genes and alleles (genotype)
• Major determinants of immune response (antigen presentation and genetic restriction)
• Analysis of immune response genes - composition, structure and function
Relevance of Immunogenetics

- **Transplant immunology** (risk management of rejection)
- **Autoimmunity** (RA, IDDM, CD, SLE, MS etc)
- **Tumour immunology** (loss of HLA)
- **Infectious disease** (HIV/AIDS, dengue etc)
- **Vaccine design** (testing and application)
- **Pharmacogenomics** (HLA associations with adverse drug reactions)
- **Regenerative medicine** *(tissue repair, stem cell therapy)*
- **Anthropology + evolutionary biology** *(population genetics)*
Human Immune Response genes in the Major Histocompatibility Complex (MHC) on Chromosome 6
MHC

• Most gene dense region of the human genome (224 gene loci in 3.6 megabases)
• Contains the most polymorphic gene locus in human genome (HLA-B has 3285 alleles)
• 40% of expressed genes involved the “immune response”
Immune response (IR) genes in MHC

- **Acquired IR** (class I HLA-A, B, C; class II HLA-DR, DQ and DP)
- **Innate IR** (HLA-A, B, C, MICA, MICB)
- **Immune receptors** (G6B, G6D)
- **Co-receptors** (BTN)
- **Regulatory receptors** (NOTCH-4, AGER)
- **Inflammation** (TNF, LTA, NkB)
- **Complement** (C2, C4B)
- **Heat shock** (HSP)
- **Transport proteins** (TAP, tapasin)
- **Signalling** (NFkB)
All intra-cellular proteins (including viruses in an infected cell) are constantly being synthesized and degraded in the cytosol.

HLA molecules are involved in antigen presentation. HLA class I molecules transport fragments (Ags) of degraded proteins to the cell surface where they are recognized by Ag-specific CD8+ T cells (CTLs).
Immunogenetics + Proteomics = “The Antigen-Binding Groove”
(see Pillars of Immunology series, J. Immunol. 174:3-5, 2005)
The HLA class I Groove has pockets (A-F)
Antigens lock into the Groove (via pockets)
MHC-peptide-TCR: Trimolecular Interaction
### HLA allelic polymorphism – January 2014

#### Number of molecularly-defined classical HLA class I alleles

<table>
<thead>
<tr>
<th></th>
<th>HLA-A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=</td>
<td>2579</td>
<td>3285</td>
<td>2133</td>
</tr>
</tbody>
</table>

#### Number of molecularly-defined HLA class II alleles

<table>
<thead>
<tr>
<th></th>
<th>HLA-DRA</th>
<th>DRB1</th>
<th>DQA1</th>
<th>DQB1</th>
<th>DPA1</th>
<th>DPB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=</td>
<td>7</td>
<td>1512</td>
<td>51</td>
<td>509</td>
<td>37</td>
<td>248</td>
</tr>
</tbody>
</table>

http://www.ebi.ac.uk/imgt/hla

www.allelefrequencies.net
There are many ways to HLA type

- HLA serology (phenotype)
- Molecular typing RFLPs (Southern blot)
- PCR-SSOP (probes)
- PCR-SSP (phototyping with primers)
- PCR-direct sequencing
- Luminex / Mutiplex (bead-based HLA typing with probes)
- 2nd generation sequencing - unambiguous resolution, high throughput
- 3rd generation sequencing – longer reads
HLA Alleles 1987 – July 2013
(www.ebi.ac.uk/imgt/HLA)

Class I alleles

Class II alleles
HLA polymorphism affects peptide binding pockets and antigen presentation
HLA polymorphism is functional

- Generated by gene duplication, recombination, inter-allelic conversion & retention of beneficial mutations
- Most of the polymorphism located around pockets A-F in Ag binding groove
- Functional implications (genetic restriction, peptide binding)
- Increased diversity of peptide binding in populations
Evolutionary significance of HLA polymorphism

• Why so polymorphic?
• Extreme polymorphism = rapidly evolving loci
• What are the selective pressures driving this polymorphism?
• Viruses, Bacteria, Parasites = Microbial pressure
• HLA diversity = historic imprint of evolution on human genome
• Natural Selection - microbes
• Species survival - adaptive immune system
• Population diversity - of immune response
• Species expansion - population increase
Screening for human genetic associations with disease

• High resolution and high throughput molecular typing now available (HUGO)
• **Candidate gene analysis** – assumes likely target worthy of detailed investigation (HLA, exons)
• **Blind association studies** – patients genome screened with large number of genetic markers (SNPs, introns, GWAS)
Strategies and variables to consider in candidate (HLA) gene association studies

- **Case-control studies**: population-based, intra- or inter-ethnic, unrelated patients and controls (ethnically and geographically matched)
- Definition of **disease phenotype** critical
- Does HLA influence **disease susceptibility** and/or severity?
- Which HLA loci, alleles, haplotypes and/or supertypes are involved?
- Methods of typing (resolution)?
- **Statistical analysis** (univariate vs multi-variate)?
- “Gold Standard” = **reproducibility** and **size** (several hundred needed for statistical power)
HLA-B51* and Bečhets Disease

- Systemic recurrent vasculitis of mucosa – oral, genital and ocular pathology
- Strong association with HLA-B*51 not B*52 (2 a.a. mm in the groove)
- Highest incidence from Mediterranean basin to Far East (the Silk Road disease)
- Linkage studies have all eventually come back to HLA-B51 - specifically B*5101 and 5108 (a.a. level)
- Unknown aetiology - response to an infectious agent - streptococci?
- **Is autoimmunity the price we pay for effective resistance to infectious disease?**
HIV/AIDS Pandemic – the story so far

- Recent zoonotic transfer (primates to man)
- Currently 35.3 million HIV infections
- 1.6 million deaths in 2012
- 2.3 million new infections in 2012
- Treatable (ARVT) and controllable disease
- Vaccines not yet?
HIV-1 Diversity

- Small RNA genome (<10Kbp)
- Extreme propensity to mutate (rapid diversification)
- "Main" or M group composed of numerous subtypes (clades A, B, C, D, F1, F2, G, H, J, K + recombinants) all circulate in African populations
- HIV-1 clade B also in Caucasoids and African-Americans (Europe, Americas, Australia/NZ) and Asians (Far East)
- HIV-1 clade C also present in S.Asians (India)
- HIV-1 intersubtype (B/C) and circulating recombinant (CRF01_AE, or Thai E) in mainland SE Asians
- Prevalence of HIV-1 clades in 2007 (40 million infections): clade C (48%), clade A (12%), clade B (11%)
HIV-1 clade distribution and prevalence 2007
(see Hemelaar in Trends in Molecular Medicine 18:182, 2012)
HIV-1 diversity in mainland SE Asians in 2000
HIV-1 diversity in mainland SE Asians in 2003/4
HIV-1 diversity versus HLA polymorphism in mainland SE Asian populations?

- Current segregation of HIV-1 clades and recombinants in large and diverse populations of mainland SE Asia, appears to correlate with major immunogenetic profiles of populations at risk of HIV-1 exposure (Trends Immunol 28: 41-47, 2005)
# Large HLA class I association studies with HIV-1

*(Infection, Genetics and Evolution. 12:1481, 2012)*

<table>
<thead>
<tr>
<th>HIV-1 Clade</th>
<th>Study Group (Location)</th>
<th>Global Prevalence</th>
<th>HLA class I associations with HIV/AIDS</th>
<th>Number of studies &gt;200 cases</th>
</tr>
</thead>
</table>
| A           | Central & East Africa  | 12.0%             | Low viral load  
Slow progression  
Low transmission  
High viral load,  
Fast progression,  
High transmission | 5 |
| B           | N. America W. Europe East Asia Oceania | 11.3% | HLA class I heterozygosity;  
A*02:05, A2 supertype, A32,  
A*33:03, B*27, B*14, B27  
supertype, Bw4, Bw4-80I,  
B*44, B*57, B*58:01, B*51,  
B*15:03, B*15:16, B*15:17,  
B*81; C*08, C*12, C*14, C*18  
HLA class I homozygosity,  
A*23, B*58:02  
A*24, B*14, B*35, B*15:02,  
B*58:01, C*06, C*07  
A*23, B*18, B*15:03,  
B*15:10, B*45; B*58:02,  
C*06, C*07 | 29 |
| C           | Central, East & South Africa | 48.2% | A2 supertype; A*0205;  
A*68:01; A*74, B*13, B*39,  
B*42, B*44, B*57, B*58:01,  
B58 supertype, B*81, C*03,  
C*04, C*12, C*18  
HLA class I homozygosity,  
A*23, B*18, B*15:03,  
B*15:10, B*45; B*58:02,  
C*06, C*07 | 13 |
| CRF01_AE    | Southeast Asia          | 5.1%              | B*13:02, B*57, B*58:01  
HLA class I homozygosity | 3 |
| CRF01_BC    | East Asia               | 1.5%              | A*33:03, A*11, Bw4/Bw6;  
B*44, B*58:01, C*04  
A*24, B*14, B*35, B*15:02,  
B*40, B*48, C*08 | 2 |
Are HLA associations with HIV-1 clade specific?

- **HLA-B*1516** and **B*1517 (B63)** associates with **low viraemia** in HIV-1 clade B but not clade A infections
- **HLA-B*1503 (B72)** associates with **low viraemia** in HIV-1 clade B but not clade C infections
- **HLA-B*18** associates with **high viral loads** in HIV-1 clade C but not clade B infections
- **HLA-B*53** associates with **high viral loads** in HIV-1 clade B but not clade A and C infections

- Implications for vaccine design?
HLA-B*57 and *58 (B58 supertype) associations with HIV/AIDS

• Most reproducible associations with low viraemia and prolonged AIDS survival in different ethnic groups (Caucasoid and African)
• Major genetic association is with HLA-B57 (not with other linked alleles)
• HIV-1 clade B and C escape mutants (avoiding HLA-57 restricted CTL responses) are replication defective (*Nature Medicine 10: 282-289, 2004*)
• HIV-1 being attenuated by HLA-B57 driven CTL?
• Implications for vaccine design?
Viruses have evolved mechanisms to interfere with the expression of HLA molecules

- **EBV** - down regulates HLA class I (*Cancer Surveys 13: 53-80, 1992*)
- **CMV** - interferes with the stability of HLA class I (*Immunity 20: 71-85, 2004*)
- **HPV** - holds class I in ER (*Int J Cancer 113: 276-283, 2005*)
- **HSV** - blocks TAP (*Nature 375: 411-415, 1995*)
Natural Killer (NK) cells and the “missing self” hypothesis

- Inhibition of cytotoxicity
- Normal MHC class I expression
- Healthy cell

- Altered expression of MHC class I
- Tumour or virus-infected cell

- Lysis
- No inhibition
- NK cell

SHP-1
Killer Ig-like receptors (KIR) recognise classical MHC class I molecules.
Central TcR recognition and distal KIR recognition of HLA class I groove
The HLA Bw4 motif
## Large NK KIR gene association studies with HIV-1


<table>
<thead>
<tr>
<th>HIV-1 Clade (Location)</th>
<th>Ethnic Group (Location)</th>
<th>KIR Locus</th>
<th>Target HLA class I allele</th>
<th>Association with HIV/AIDS</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Caucasian (Oceania)</td>
<td>2DS2</td>
<td>HLA-C* gp 1 alleles (77Ser,80Asn)</td>
<td>2DS2 copy number</td>
<td>Genes Immun 6:683, 2005</td>
</tr>
<tr>
<td>B</td>
<td>Caucasian (Oceania)</td>
<td>2DL3</td>
<td>HLA-C* gp 1 alleles (77Ser,80Asn)</td>
<td>2DL3</td>
<td>Genes Immun 6:683, 2005</td>
</tr>
<tr>
<td>C</td>
<td>African (Sub-Saharan)</td>
<td>2DS4</td>
<td>HLA-C* gp 2 alleles (77Asn,80Lys)</td>
<td>2DS4*001</td>
<td>JID 203:487, 2011</td>
</tr>
</tbody>
</table>
Genome wide association studies (GWAS) with HIV-1

- 9 studies performed to date
- 1000’s patients analysed
- 300,000-1,300,000 SNPs on Illumina platform
- European, African-American, Hispanic patients (clade B)
- Africa/Asia?
HLA associations with HIV-1 viral load confirmed or replicated by numerous SNP genome scans (PLoS ONE, Dec 2008, Vol 3)
Amino acid associations with HIV-1 from deduced HLA class I types
(HLA-B57 only allele with Val at pos 97)

Science 2010;330:1551-1557

Published by AAAS
Fig. 4 Three-dimensional ribbon representation of the HLA-B protein based on Protein Data Bank entry 2bvp (30), highlighting amino acid positions 62, 63, 67, 70, and 97 lining the peptide binding pocket.
Phase 3 HIV Vaccine Field Trials

• 6 trials performed to date
• 5 trials no efficacy (Env gp120 protein or adenovirus expressing Gag, Pol, Env and Nef)
• 1 trial in Thailand has shown some efficacy
• Prime: (ALVAC) vector (canary pox) with gp120 (clade A/E + B), gp41 (clade B), gag + protease (clade B)
• Boost: (AIDSVAZ): protein gp120 (clade A/E)
RV144 HIV Vaccine Field Trial in Thailand
(see NEJM 361:2209, 2009)

• Phase 3 HIV/AIDS vaccine efficacy field trial in Rayong / Chon Buri (randomised, double-blind, placebo controlled)
• 16,000 volunteers, low prevalence (0.3% incidence/yr)
• Vaccine designed to invoke neutralising Ab responses to gp120 and CTL Gag, Pol and Env in recipients.
• After 42 months HIV-1 incidence in vaccine gp = 0.68% vs 0.96% with placebo Vaccine efficacy = 31.2%
• Not good enough to license
• Proof of concept “Vaccine prevention of HIV is possible”
• Highly informative, correlates of protection?
RV144 – post hoc analysis

• Vaccine efficacy 68% in low risk group but only 5% in high risk group
• IgG Abs to variable regions of Env V1/V2 envelope contribute to protection (OR = 0.57)
• IgA to Env gp120 correlate with increased rate of infection (OR = 1.54)
• Vaccine-induced genetic signatures in breakthrough HIV-1 isolates
• RV144 elicits protective immune responses in some individuals against some isolates
Is there a role for HLA in the RV144 Vaccine Story? 
*(see Vaccine 30: 832-836, 2012)*

<table>
<thead>
<tr>
<th>HLA class II allele</th>
<th>Neut Ab producer</th>
<th>Neut Ab non-producer</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DRB1*11</strong></td>
<td>9%</td>
<td>54%</td>
<td>0.09</td>
<td>0.02-0.44</td>
<td>0.0021</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>DRB1*16</strong></td>
<td>0</td>
<td>31%</td>
<td>0.05</td>
<td>0.002-0.92</td>
<td>0.0059</td>
<td>0.076</td>
</tr>
<tr>
<td><strong>DQA1<em>05:01 + DQB1</em>03:01</strong></td>
<td>7.8%</td>
<td>30.8%</td>
<td>0.19</td>
<td>0.05-0.75</td>
<td>0.009</td>
<td>0.180</td>
</tr>
</tbody>
</table>
HLA-B57 and Abacavir-induced Type B adverse drug reactions (ADR)

- RTase inhibitor: nucleoside analogue, ART (HIV/AIDS)
- Hypersensitivity: 8% of recipients (ethnicity?)
- Re-challenge: hypotension, morbidity, mortality
Known HLA associations with Adverse Drug Reactions

- **Drug induced hypersensitivity**: Abacavir (B*57:01), Allopurinol (B*58:01), Nevarapine (DRB1*01:01, B*35:05, C*08), Dapsone (B*13:01)
- **Toxic epidermal necrolysis (TEN) / Steven-Johnson Syndrome (SJS)**: Allopurinol (B*58:01), Carbamazepine (B*15:02), Lamotigine (B*38), Phenytoin (B*15:02), sulphonamides (DRB1*07:01)
- **Drug induced liver injury (DILI)**: Amoxicillin (DRB1*15:01), Flucoxacillin (B*57:01), Xielagatran (DRB1*07, DQA1*02)
- **Agranulocytosis**: Clozapine (B*38,DR4, DQw3), Levamisole (B*27)
- **Drug-induced lupus erythematosis**: Hydralazine, procainamide, isoniazid etc (DRB1*04)
Why HLA and idiosyncratic Adverse Drug Reactions?

- **Multifactorial:** combination of immune response, infections, metabolic products, genetic predisposition
- **Danger signal:** oxidative stress, trauma/surgery, infection, pro-inflammatory responses trigger and immune response to drug
- **Hapten hypothesis:** host proteins modified by drug metabolites (processed as Ag by HLA)
- **Pharmokinetics:** drug acts on HLA and/or Tcell directly
- **Altered peptide ligand:** Abacavir affects Ag binding groove of B57
Altered peptide binding of HLA-B57 induced by the anti-retroviral Abacavir (Nature 486: 479 and 554, 2012)
Immunogenetic Surveillance of HIV/AIDS

(see Infection, Genetics and Evolution 12: 1481-91, 2012)

- HIV-1 recent pandemic
- Selective force on human gene pool
- >50 large studies reproducing HLA associations with pathology in 1000’s of patients
- Meta analysis confirm strong association with HLA genes, alleles, function
- Understanding protective immune responses
- Relevance to design and testing of new vaccines to prevent HIV-1 transmission
Immunogenetic Surveillance

• Field studies now incorporating HLA typing of at risk populations in initial epidemiological screen (complement virus surveillance networks)
• Work with local labs and utilize their expertise
• Know your populations (ethnicity, genetic profiles)
• Investigative and empirical approach, not experimental or animal model driven
• Relatively low cost and high data or information output
• Integrative, well suited to multidisciplinary research programmes
• Integral component of vaccine design and testing
Collaborating Institutes

**Transfusion Medicine - Siriraj Hospital:** S. Vejbaesya, D. Chandanayingyong

**Retrovirology – AFRIMS / WRAIR:** A. Brown, R. Paris

**Johns Hopkins University:** C. Beyrer, K. Nelson

**CDC Atlanta** T. Mastro, K. J. McNicholl, T. Hodge

**Guy’s Hospital:** R. Vaughan, R. Collins

**Stanford:** P. Norman

**UCL CFN & Anthony Nolan Labs:** A. Karasu

**Funding:** NIH, NRC, WRAIR, UCLH and RFH Trusts