

**2003**

**7<sup>th</sup> Asia-Oceania Histocompatibility  
Workshop and Conference  
Abstracts**



## Symposium

### (S1-2) The evolution of the MICA and HLA-B polymorphisms

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The MICA locus codes for a stress inducible surface glycoprotein which is expressed in gastrointestinal epithelium and some epithelial tumors. The MICA molecules are — in contrast to regular class I molecules — not associated with  $\beta_2$ -microglobuline and they do not appear to bind specific peptides. The MICA molecule binds with the natural killer receptor NKG2D and co-stimulates some  $\gamma$ - $\delta$ T-cells as well as antigen specific CD8  $\alpha$ - $\beta$  T-cells. The restricted tissue distribution and the inducibility by cytomegalovirus has raised the speculation that MICA gene products could be involved in graft-versus-host disease. In addition it has become obvious that the interaction of MICA with NKG2D could provide stimulation of cytotoxicity superseding stimulation or inhibition of NK cells in the defense against neoplasia.

We have developed techniques for the detection of polymorphisms in exons 2, 3, 4 and 5 as well as in the introns. Exons 2, 3, 4 and 5 were separately sequenced. Using the sequence data base for MICA alleles assembled by Steven Marsh, we were able to include the MICA exons 2, 3, 4 and 5 polymorphism in the Helmborg Score Computer Analysis Program. We have investigated the relationship between the polymorphisms of MICA and HLA-B in a variety of samples from the International Workshop cell lines, from Thailand, from China, from Brazil, from North Africa and from the International Cell Exchange, UCLA as well as from our panel and families in our laboratory. The aim of the study is to correlate HLA-B alleles (sequenced for exon 2 and 3) with MICA alleles sequenced for exons 2, 3, 4 and 5 with a particular emphasis on the subtypes in the HLA-B allele groups as for example B\*3501, \*3503, \*3504, \*3505, \*3511 and \*3512. The combined sample size is greater than 350.

The following results became apparent:

- 1) There is very strong linkage disequilibrium between HLA-B and MICA as would be expected. This linkage disequilibrium extends over widely differing populations around the globe.
- 2) The most common MICA allele \*008 is associated with a number of HLA-B alleles (B\*07, \*08, \*4402).
- 3) Several groups of closely related B locus alleles are strongly (almost exclusively) associated with the same MICA allele: B\*38, \*39 with MICA\*002; B\*51, \*52 with MICA\*009; B\*54, \*55, \*56 with MICA\*012.
- 4) Many B locus subtypes of B\*35 or of B\*38, \*39 share the same MICA association.

The fact that the various subgroups of HLA-B originating in widely differing populations share the same MICA allele indicates, that the MICA polymorphism is more ancient than HLA-B, or in other words HLA-B is evolving more rapidly than MICA.

### (S1-3) Diversity of Immune Loci: Lessons from Genome Sequencing

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The Human Genome Project is probably the most important project in modern biology with far-reaching implications for biomedical research including immunology. A free 'working draft' sequence has been publicly available since June 2000 and an essentially finished sequence since April 2003. The on-going analysis and annotation of the human genome have been particularly enhanced through recent 'working draft' sequences of the mouse and several fish genomes. The visualization of this great wealth of diverse genome data has been (and still is) a great challenge for bioinformatics and resulted in the development of the ENSEMBL genome database at <http://www.ensembl.org/>, the UCSC genome browser at <http://genome.ucsc.edu/> and various other comparative analysis tools.

The lecture will include a brief history of these projects and review some of the key lessons learnt from the sequence so far. These will include excursions into comparative genomics and functional genomics and will be discussed in the context of diverse immune loci

including those of the major histocompatibility complex (MHC) and leukocyte receptor complex (LRC).

#### **(S1-4) Genetic diversity and genomics of the immune response: identifying immune variation within the MHC and throughout the genome**

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With the advent of modern genomic sequencing technology the ability to obtain new sequence data and to acquire allelic polymorphism data from broad range of samples has become routine. In this regard, our investigations have started with the most polymorphic of genetic regions fundamental to the immune response in the MHC. Starting with the completed human MHC genomic sequence, we have developed a resource of methods and information that provide ready access to a large portion of human and nonhuman primate MHCs. This resource consists of a set of primer pairs or amplicons that can be used to isolate about 15% of the 4.0 Mb MHC. Essentially similar studies are now being carried out on a set of immune response loci to broaden the usefulness of the data and tools developed. A panel of 100 genes involved in the immune response have been targeted for SNP discovery efforts that will analyze 120 Mb of sequence data for the presence of immune-related SNPs. We have used this approach to analyze a large number of diverse human haplotypes and have extended analysis to non human primates represented by the gorilla, baboon, macaque, and marmoset. The SNP data provided from the MHC and from the immune response panel has been adapted for use in studies of evolution, MHC disease associations, and clinical transplantation.

#### **(S1-5) From HLA to genome-wide scan: common disease gene mapping by association analysis using microsatellites**

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Now that human genome sequencing has been completed, it is an upcoming important subject to carry out identification of disease genes, especially for common diseases such as schizophrenia, atopy, diabetes, hypertension, cerebral infarction, cardiac failure etc) which have multifactorial genetical basis. This “human genome diversity project” is conducted by mapping and association analyses using polymorphic genetic markers. For this purpose, SNP (single nucleotide polymorphism) markers are now being extensively and world-widely collected, and applied to disease mapping in a lot of laboratories. However, SNP is generally bi-allelic, and so polymorphism is not considered to be extensive enough to localize disease genes on the human genome by genome-wide mapping.

Instead, we propose to use microsatellite which displays a high degree of polymorphism in repeat number of repetitious unit and so is expected to serve as a more useful genetic marker for genome-wide mapping. To test this hypothesis, we have focused on mapping of diseases in the HLA region on chromosome 6p21.3, which is divided into class I, class II and class III regions from centromere to telomere. The human MHC (HLA) encompasses a 3.6 Mb (3,600 kb) segment, which is characterized by high gene density (one gene/18 kb), extensive genetic polymorphism (more than 900 alleles at 12 HLA loci) and the presence of susceptible loci for more than 100 diseases (HLA-associated diseases). In these respects, the HLA region provides an excellent mini-genome model region for “human genome diversity project”. This area has been determined for the complete genomic sequence by the international consortium groups (1). Among these efforts, our group (Tokai University) determined the nucleotide sequence of the 2.2 Mb continuous HLA region including the 1.8 Mb entire class I region as well as the 0.4 Mb class III region (2), in order to elucidate the detailed gene organization in the HLA class I region and identify susceptible genes for many HLA class I-associated diseases .

A total of 1502 microsatellite repeats were identified in the 3.6 Mb HLA genomic sequence. These consist of 409 di-, 266 tri-, 539 tetra- and 307 penta-nucleotide repeats, yielding an overall density of one microsatellite per 2.4 kb. Among the 1502 microsatellite identified here, 144 have been selected and subjected to polymorphism analysis within a Japanese population. As expected, 78 out of these 144 microsatellites are quite polymorphic with an average of 10.0 alleles and 62.9% heterozygosity. As these polymorphic microsatellites are evenly dispersed throughout the HLA region, they should serve as much needed genetic markers in linkage and association analyses, enabling investigators to precisely map HLA-associated disease susceptibility loci. In fact, by investigation of genetic polymorphisms in 78 microsatellite repeats, we could successfully reduce the critical regions for Behcet’s disease (associated with B51) to a 100 kb segment around the HLA-B gene, for psoriasis vulgaris (with Cw6) to a 50 kb segment telomeric of HLA-C, and for rheumatoid arthritis (the second susceptible locus in the HLA region) to a 50 kb segment between IkbL and MICB. From these segments, susceptible genes for those three diseases, HLA-B51 for Behcet’s disease, SEEK1 for psoriasis vulgaris and IkbL for rheumatoid arthritis were identified from these critical candidate regions by SNP association analysis.

Thus, refined microsatellites provide useful and valuable genetic markers for precise disease mapping. In contrast, SNP mapping are not

easy even for these HLA associated diseases at the Mb level mapping, mainly due to a few alleles (two alleles) and low heterozygosity of SNP. These results are validated by different lengths of linkage disequilibrium observed for SNP and microsatellite. Namely, the length of linkage disequilibrium observed for SNP is less than 5 kb, whereas it is as long as 100 kb for microsatellite. This suggests that one microsatellite per 100 kb is enough for genome-wide mapping. Collectively, the efficient method of genome-wide mapping is to first use microsatellites markers which enable to narrow down the critical region to approximately 100 kb and thereafter to employ SNP markers within thus determined critical region for fine mapping to identify a susceptible gene. Based on this conclusion, we have collected 30,000 polymorphic markers (one microsatellite per 100 kb) throughout the human genome which will be subjected to genome-wide mapping of complex diseases. Now we are conducting genome-wide association mapping of 20 common diseases using 30,000 microsatellites.

#### References

- (1) The MHC sequencing consortium: Complete sequence and gene map of a human major histocompatibility complex (MHC). *Nature* 401: 921–923, 1999.
- (2) Shiina T, Tom G, Oka A, Takishima N, Yamagata T, Kikkawa E, Iwata K, Tomizawa M, Okuaki N, Kuwano Y, Watanabe K, Fukuzumi Y, Itakura S, Sugawara C, Ono A, Yamazaki M, Tashiro H, Ando A, Ikemura T, Soeda E, Kimura M, Bahram S, Inoko H : Molecular dynamics of MHC genesis unraveled by sequencing analysis of the 1,796,938 bp HLA class I region. *Proc Natl Acad Sci USA* 96 : 13282–13287, 1999.

### (S1-6) Immunogenetics of non-conventional MHC class I genes.

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The past century has witnessed the unfolding of perhaps the most formidable saga of human genetics and biology. First identified genetically and serologically, then characterized biochemically, and finally defined at the nucleotide and structural levels, the Major Histocompatibility Complex (MHC), approximately one-thousandth of the genome, encompasses its most polymorphic members. This diversity enables the MHC to counter-act the extraordinary diverse microbiological threats and, paradoxically, also engenders the not well understood susceptibility to a large number of pathologies. The MHC is defined by two gene classes, I and II, which are structurally related but functionally distinct, each displaying a different set of peptide antigens to  $\alpha\beta$  T-cell receptor expressing killer and helper T cells respectively. Class II molecules are restricted to the MHC proper, located on the sixth chromosome in man, but the MHC-I molecules are dispersed throughout the genome and accomplish a variety of unrelated tasks (Figure 1). This lecture will review our current knowledge of the functional genomics of the MHC with special emphasis on the large number of MHC class I genes identified outside the MHC per se on chromosome 6p21.3. Wherever applicable, reference will be made to genetic diversity within these loci as well as to their functional relevance in disease susceptibility.

#### Further readings:

- Bahram, S. 2000. MIC genes: from genetics to biology. *Adv Immunol* 76:1.
- Bendelac, A., M. Bonneville, and J. F. Kearney. 2001. Autoreactivity by design: innate B and T lymphocytes. *Nat Rev Immunol* 1:177.
- Garboczi, D. N., and W. E. Biddison. 1999. Shapes of MHC restriction. *Immunity* 10:1.
- Hill, A. V. 1998. The immunogenetics of human infectious diseases. *Annu Rev Immunol* 16:593.
- Kronenberg, M., Gapin, L. 2002. The unconventional lifestyle of NKT cells. *Nat Rev Immunol*. 2:557.
- Porcelli, S. A., and R. L. Modlin. 1999. The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* 17:297.
- Radosavljevic, M. and Bahram, S. 2003. *In vivo* immunogenetics: from *MIC* to *RAET1* loci. *Immunogenetics* 55: 1.
- Wilson, I. A., and P. J. Bjorkman. 1998. Unusual MHC-like molecules: CD1, Fc receptor, the hemochromatosis gene product, and viral homologs. *Curr Opin Immunol* 10:67.

### (S1-7) Genetic and functional relationships between MHC and NK receptor genes and their products.

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Over 5% of the genes in the human genome are involved in defence against infection. Some of these genes have features consistent with this role. For example, many of them are polygenic and polymorphic. They tend to be clustered in the genome. Some are evolving rapidly, by allele or gene conversion. We are studying some of these immune system genes to determine their role in disease resistance. Our main focus includes the MHC and NK complexes as the extensive variation in both MHC and NK receptor loci may reflect continuous selection for resistance to pathogens. And, since the genes are on different chromosomes it is expected that there may be epistatic interactions between products of alleles from class I and NK receptors. In collaboration with other groups, including Mary Carrington's laboratory at

Frederick, we are obtaining evidence for these interactions in MHC class I-associated diseases.

## (S2-2) A structural basis for the selection of dominant alpha/beta T cell receptors in anti-viral immunity

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We have examined the basis for immunodominant or “public” TcR usage in an antiviral CTL response. Residues encoded by each of the highly selected genetic elements of an immunodominant clonotype recognising Epstein-Barr virus were critical to the antigen specificity of the receptor. Upon recognising antigen, the immunodominant TcR undergoes extensive conformational changes in the complementarity determining regions (CDRs), including the disruption of the canonical structures of the germline-encoded CDR1 $\alpha$  and CDR2 $\alpha$  loops produce an enhanced fit with the HLA-peptide complex. TcR ligation induces conformational changes in the TcR  $\alpha$  constant domain thought to form part of the docking site for CD3 $\epsilon$ . The structure of a CD3 $\epsilon$ g dimer complexed with a Fab of OKT3 provides further evidence for this model. These findings indicate that TcR immunodominance is associated with structural properties conferring receptor specificity and suggest a novel structural link between TcR ligation intracellular signaling.

## (S2-3) DOCK2, CED-5 human homologue, controls mobility and function of lymphocytes

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Cell migration is a fundamental biological process involving membrane polarization and cytoskeletal dynamics. Cell migration is crucial in embryogenesis, but it also plays an important role in physiological function of the adult organism. The typical example is seen in the immune system, where lymphocytes continually patrol the body for foreign antigens. T- and B- lymphocytes differentiated in the primary lymphoid organs, thymus and bone marrow, are released into peripheral blood and migrate to particular sites of the secondary lymphoid organs such as spleen, lymph nodes and Peyer’s patches to elicit antigen-specific immune response. It is well established that lymphocyte migration is initiated and guided by chemokines. However, very little is known about the molecular mechanism that regulates lymphocyte motility itself.

The CDM family proteins, CED-5 in *Caenorhabditis elegans*, Myoblast City in *Drosophila melanogaster* and DOCK180 in humans, are known to mediate remodeling of actin cytoskeleton. We have identified a new member of the CDM family protein DOCK2 that is predominantly expressed in lymphocytes. DOCK2-deficient mice exhibited migration defects of T and B lymphocytes in response to chemokines, resulting in several abnormalities including T lymphocytopenia, atrophy of lymphoid follicles and loss of marginal zone B cells. In DOCK2<sup>-/-</sup> lymphocytes, chemokine-induced Rac activation and actin polymerization were totally abolished. These results indicate that DOCK2 plays a central role in lymphocyte migration by remodeling actin cytoskeleton though Rac activation. In addition, we recently found that DOCK2 also functions downstream of T cell receptor and regulated antigen-recognition. Therefore, DOCK2 might be a novel therapeutic target for graft rejection and autoimmune diseases.

## (S2-4) HLA associated genetic predisposition to autoimmune diseases

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Autoimmune diseases are the result of an interplay between genetic predisposition and precipitating environmental factors. While the environmental factors are largely unknown, more are known about the predisposing genes. Genes in the HLA complex are by far the strongest predisposing genes, and account for approx. 50% (or more) of the genetic predisposition. A large body of evidence strongly suggests that genes encoding particular peptide-presenting HLA class I or II molecules are the strongest predisposing genes.

Recent studies have shown, however, that also other genes among the approx. 130 genes in the HLA complex play an important role. Using appropriate methods to control for the strong linkage disequilibrium between alleles in the HLA complex, we have been able to demonstrate that several additional HLA regions contain genes which independently contribute to the HLA associated genetic predisposition. This was further corroborated in the studies conducted by the Disease Component of the recently finished 13. International

Histocompatibility Workshop. The nature of these additional HLA complex genes are currently under investigation, and may be common predisposing genes for several autoimmune diseases.

The identification of genes predisposing to autoimmune diseases will lead to better methods to identify individuals at high risk to develop these diseases. When the function of these genes has also been unveiled, this may lead to better methods to treat and prevent these diseases from developing in those at high risk.

### **(S2-5) Manipulation of immune response by genetically modified dendritic cells derived from mouse ES-cells**

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Dendritic cell (DC)-based immunotherapy is regarded as a promising means for anti-cancer therapy. The efficiency of T cell-priming *in vivo* by transferred DCs should depend on their encounter with T cells. In the present study, we attempted to improve the capacity of DCs to prime T cells *in vivo* by genetic modification to express chemokine with T cell-attracting property. For genetic modification of DCs, we used a recently established *in vitro* method to generate dendritic cells from mouse embryonic stem (ES) cells (ES-DC). After stimulation of ES-DC with IL-4 plus TNF- $\alpha$ , combined with anti-CD40 mAb or LPS, ES-DCs expressed MHC class II, CD11c, CD80, and CD86, and completely became mature DCs, characterized by a typical morphology and higher capacity to stimulate primary MLR. Using an expression vector containing the internal ribosomal entry site (IRES)-puromycin N-acetyltransferase gene, we could efficiently generate ES cell transfectants expressing the products of introduced genes after their differentiation to DCs. ES-DCs expressing invariant chain-replaced by a PCC epitope at CLIP region presented the epitope efficiently in the context of E<sup>k</sup>. We primed OVA-specific and MHC class I-restricted cytotoxic T lymphocytes *in vivo* by injecting mice with ES-DCs expressing OVA, thus demonstrating immunization with ES-DCs genetically engineered to express antigenic protein. Furthermore, we generated double-transfectant DCs expressing a chemokine along with a model antigen, OVA, by sequential transfection of ES cells then inducing differentiation to DCs. Immunization with DCs expressing OVA plus secondary lymphoid tissue chemokine (SLC) or monokine induced by IFN- $\gamma$ (Mig) provided protection from OVA-expressing tumor cells more potently than that with OVA only, and SLC was more effective than Mig. The findings provide useful information for the development of a potent DC-based cellular immunotherapy for cancers. The attempt to inhibit antigen-specific response of autoreactive T cells by using ES-DCs expressing a specific autoantigen together with immunosuppressive molecules is also ongoing to prevent development *in vivo* of an experimental autoimmune disease in mice.

### **(S2-6) NK receptor genes are predictors of HIV progression**

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NK cell receptors and HLA molecules are intimately involved in the immune response against viral infections. The killer immunoglobulin-like receptors (KIRs) on NK cells regulate responses via the recognition of their particular HLA class I ligands. At the genomic level, both genetic systems are polymorphic and have recognised haplotypes, which adds complexity to their interaction. Previously, we and others have shown the association of HLA class I with HIV progression. More recently, the Carrington group observed a synergistic effect involving the activating KIR3DS1 gene and HLA-B Bw4-80Ile on the progression to depletion of CD4+ T cells.

To further investigate the relationship between the two genetic systems, we examined changes in viral load in 249 pre-treatment patients from the Western Australia HIV cohort. Genetic variants known to affect HIV progression but not KIR3DS1 were associated with changes in HIV-1 viral load. When we examined only those patients with known seroconversion dates, we found that the presence of the KIR3DS1 gene was associated with more rapid decline in the proportion of CD4+ T cells ( $p = 0.01$ ) but were unable to show a KIR3DS1 and Bw4-80Ile interaction. The presence of other KIR genes (*viz* KIR2DS2, KIR2DL2 and KIR2DS1), all found on KIR B group haplotypes, were also associated with a more rapid progression.

We suggest that KIR genes influence outcome in HIV infection and that further analyses of haplotypes in both genetic systems is needed to understand their complex interactions in driving NK cell responses to HIV.

### **(S2-7) HIV-1 escape from HIV-1-specific CD8 T cells in individuals with HIV-1 infection**

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HIV-1-specific CD8<sup>+</sup> T cells play a critical role in the control of human immunodeficiency virus type-1 (HIV-1) infection. CD8<sup>+</sup> T cells

inhibit the replication of HIV-1, not only by cytolytic mechanisms but also by the release of HIV-suppressive factors such as interferon- $\gamma$  (IFN- $\gamma$ ) and chemokines. However, although HIV-1-infected individuals exhibit a strong HIV-1-specific cytolytic response, these individuals usually develop AIDS if they are not treated with anti-retrovirus therapy. These findings indicate that HIV-1 can escape from HIV-1-specific CD8<sup>+</sup> T cells. The HIV-1 escape may occur via different mechanisms such as mutations of immunodominant epitopes, apoptosis of HIV-1-specific CD8<sup>+</sup> T cells via Fas and TNF, impaired cytolytic function of HIV-1-specific CTLs and Nef-mediated HLA class I down-regulation.

To investigate the mechanism of HIV-1 escape from HIV-1-specific CD8<sup>+</sup> T cells, we studied recognition of HIV-1-specific CD8<sup>+</sup> T cells for wild type (Nef-positive; Nef<sup>+</sup>) HIV-1- or Nef-defective HIV-1-infected CD4 T cells using HIV-1-specific CD8 T cell clones. Most CTL clones failed to kill Nef<sup>+</sup> HIV-1-infected CD4 T cells but partially inhibited Nef<sup>+</sup> HIV-1 replication. These CTL clones were capable of producing cytokines after recognizing Nef<sup>+</sup> HIV-1-infected CD4 T cells, implying that the cytokines secreted from HIV-1-specific CTL clones play an important role in inhibition of HIV-1 replication. These findings may account for the fact that HIV-1-specific CD8<sup>+</sup> T cells partially inhibit HIV-1 replication in chronically HIV-1-infected individuals. We further analyzed HIV-1-specific CD8<sup>+</sup> T cells in patients with acute and chronic HIV-1 infection using HLA class I tetramers which were generated using more than 20 HIV-1 epitopes. *Ex vivo* analysis of the peripheral blood demonstrated that CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>-</sup> memory/effector subset predominantly increased in HIV-1-specific CD8<sup>+</sup> T cells in patients with acute and chronic HIV-1 infection. This is in contrast to the fact that CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>-</sup> and CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+</sup> effector subsets predominantly increased in HCMV-specific CD8<sup>+</sup> T cells in both HIV-1 infected and healthy individuals. Function of CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>-</sup> HIV-1-specific CD8<sup>+</sup> T cells will be discussed.

## (S2-9) Immunobiology of natural killer cells mediated by activating and inhibiting receptors

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Natural Killer (NK) cells recognize abnormal cells such as transformed tumor cells, virus infected cells, and cells undergoing stress. Studies using tumor models have demonstrated that NK cells become activated upon interaction with target cells that have lost expression of “self” MHC class I antigens i.e. the missing “self” recognition. Malignant and virally transformed cells may, however, upregulate ligands for the activating receptor NKG2D and override inhibitory signals mediated by self-MHC. Alternatively, target cells may lose MHC class I expression and thereby induce NK activation. It is therefore currently not known how concomitant inhibitory and activating signals are coordinated to regulate NK effector function. The inhibitory KIRs, 2DL2 (CD158b1) and 2DL3 (CD158b2) have ligand specificity for HLA-Cw antigens of the Cw3 group and 2DL1 (CD158a) has ligand specificity for the Cw4 group. Accordingly, KIR2DL receptors cover the complete spectrum of HLA-Cw antigens in the human population and constitute one receptor-ligand system for tolerance to “self” and protection against auto-aggression. Other inhibitory receptors for HLA class I molecules include 3DL1 (ligand specificity for Bw4), ILT2 (ligand specificity for multiple HLA class I) and CD94/NKG2A (ligand specificity for HLA-E). All inhibitory NK receptors mediate their inhibitory function by recruitment and activation of cytoplasmic tyrosine phosphatases to the immunoreceptor tyrosine-based inhibitory motif (ITIM) within the cytoplasmic domain of the receptor. In the absence of inhibitory signals, NK cells are activated by ligand interactions with activating receptors such as natural cytotoxicity receptors (NCR), activating KIRs, NKG2D, CD94/NKG2C, 2B4 and others.

We will present data on mechanistic aspects on NK cell inhibition obtained from analysis of the Natural Killer Cell Immune Synapse (NKIS). It is demonstrated that inhibitory KIR provides a scaffold for ligand-induced translocation of inhibitory signals into the central supramolecular inhibitory cluster resulting in interruption of activation pathways.

Secondly, we will demonstrate how the KIR genotype for inhibitory receptors influences the outcome of allogeneic hematopoietic stem cell transplantation (HCT). This effect is observed also in HCT with HLA genotypically identical sibling donors. The effect of KIR mismatching is observed in patients with acute myelogenous leukemia (AML) and is demonstrated by reduced rate of leukemia relapse. Finally, we will demonstrate an example of tumor evasion from NK cell mediated immune responses. NK cells from patients with colorectal cancer frequently lack expression of activating NKG2D and chemokine CXCR1 receptors, both of which are internalized. Serum levels of NKG2D ligands, MHC-class-I-chain-related (MIC) molecules are elevated and are responsible for downmodulation of NKG2D and CXCR1. Our studies indicate that MIC-expressing tumor cells evade NK cell immunity by deactivating NK cells via the soluble MIC-NKG2D interaction.

These studies provide examples of NK cell biology and effector functions in health and diseases. The studies illustrate that NK cells play important roles in immune responses and are key effector cells in immunosurveillance of malignant cells.

### **(S3-1) Origin & implications of genomic diversity in disease susceptibility, in Southern India.**

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Some of the grandest experiments of Nature has been conducted in the name of Caste system in southern India (Dobzhansky 1973). Modern man migrated to India and farther, since the first coastal migration 50,000 years ago. A large-scale expansion in Middle East and Central Asia and search of new pastures resulted in emigration to India: Many of these populations formed ancient settlers in India. Ten thousand years ago, Fertile Crescent, Middle East, Baluchi-Harappan tradition, (7000BC to 2000BC), and the whole of India were occupied by people speaking Dravidian language in addition to other tribal and other languages. As on date, Southern India is characterized by Dravidian language family, Dravidian kinship and caste system, the sub-divided gene pools (breeding isolates). Originating in different ancient settlements they flew in, and expanded though sympatrically isolated. Thus 'Dravidian' became a culture and a linguistic family, adopted by 95% of the people of southern India. The genomic diversity thus generated in the name of sub-divided gene pools, provide us a means to study the role of MHC in the generation of immune-repertoire and differential susceptibility to various infectious diseases: it provides us an opportunity to evaluate and distinguish migration vs. selection operating on the highly polymorphic MHC region. Well-defined breeding isolates of southern India differs in their origin, migration and MHC diversity. A gradient of HLA DRB1\* alleles and MHC haplotypes from Eastern Europe to South East Asia attributed to migrations follows that pattern of NRY flow. Selection still operates on MHC loci. Disease association studies, community genetic studies, Genome Scan studies all reiterate the importance of samples identity, even in this genomic era. Studies on South Indian samples have identified high-risk alleles for tuberculosis and leprosy, genomic regions predisposing for leprosy and the role of epidemiology / vaccination status in immune repertoire at the TCR level. The genetic and immunological basis of multi-factorial, polygenic, infectious and autoimmune diseases and disorders thus need to be interpreted in the context of migrational history community genetics to obtain unequivocal results: this adds a new dimension to genomic diversity.

### **(S3-2) HLA Polymorphism and Evolutionary Inferences**

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The analysis of HLA sequence diversity can be informative with respect to the evolution of HLA polymorphism as well as the evolution of the human species. Inferences about the more recent relationships among human populations can be made by analyzing the distribution of HLA alleles in various human populations. Such population data can also be analyzed by the Ewens-Watterson homozygosity test to reveal the nature of the selective pressures that have shaped these frequency distributions. Phylogenetic analyses of HLA sequences from a variety of primate species by a variety of labs have indicated the presence of ancestral polymorphisms (i.e. predating the hominoid divergence, 5–7 myr ago) at most loci. However, most DPB1 sequences cluster by species, suggesting that this locus has been evolving faster than the other loci and/or that only one or two ancestral alleles have been retained by the modern hominoid species. Several years ago, Bergstrom et al. (Nature Genetics, 1998) compared phylogenetic trees for human DRB1 sequences from exon-2 and introns 1 and 2 and concluded that, while the major allelic lineages predate the hominoid divergence, the alleles within a lineage are of more recent origin. This interpretation of the DRB1 intron sequence data, unlike interpretations based on exon-2 sequence data, which is known to be under selection, allowed estimates of the size of and coalescence time for the founding human population that were consistent with estimates from mtDNA and other autosomal markers. We have recently used allele frequency data from a variety of human populations, using Nei's genetic distance and neighbor-joining algorithms, to construct population "networks". Although some graphical representations of these population relationships (ie dendrograms) look like sequence "trees", these population-based networks differ from gene genealogy trees based on sequence divergence in a number of fundamental ways, such as the meaning of branch length. The relationships among human populations can be inferred from such networks, and specific anthropological hypotheses (e.g .the colonization of Polynesia) can be examined. We will present the analyses of population data generated in our lab as well as data generated, using our immobilized probe HLA class I typing reagents, by participating labs in the 13th International Histocompatibility Workshop.

Reference:

Bergstrom, TF, Agnetha, J., Erlich, HA, Gyllensten, U. 1998. Recent origin of HLA-DRB1 alleles and implications for human evolution. Nature Genetics 18: 237–242.

**(S3-3) MHC class I gene organization in primates**

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**ABSTRACT** The genealogical relationships of MHC class I genes between humans and New World Monkeys (NWMs) are examined by means of SINE/LINE markers and genomic DNA sequences. From three subfamilies in NWMs (Aotinae, Atelinae, and Cebinae), 18 MHC-like genes are found. Of these, two pairs of genes are clearly orthologous to HLA-E and -F, respectively. In the remaining 14 genes, eight belong to a cladistically distinct group B, including HLA-B/C, and they are phylogenetically clustered into four clades (B1, B2, B3 and B4). The presence of group B genes in NWMs is contrary to the previous finding that there are no genes in subfamily Callitrichinae which share direct common ancestors with HLA-B/C. Furthermore, in each of B1, B2 and B3 clades, the orthologous relationships are found among different subfamilies.

This is in sharp contrast to the genus-specific gene organization in Callitrichinae. The other six genes belong to a cladistically distinct group G and are phylogenetically clustered into two clades (G1 and G2). However, since these genes are almost equally related to HLA-G, -A/H, -J and -K, it is likely that they are not orthologous to HLA-G. Since most class I genes in humans and NWMs were generated prior to the emergence of simian primates, gene duplication took place frequently during the early evolution of primates and the class I gene organization has since been somehow stabilized. The present repertoire of simian primate MHC loci has been shaped mainly by loss of loci.

**(S3-5) Evolutionary origin of the central nervous system-related genes and its implication to the MHC gene evolution**Katsuhiko Mineta<sup>1,2</sup>, Masumi Nakazawa<sup>2</sup>, Francesc Cebria<sup>3</sup>, Kazuho Ikeo<sup>1,2</sup>, Kiyokazu Agata<sup>3</sup>, and Takashi Gojobori<sup>1,2</sup>

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In the bilateral animals, the centralized nervous system was found in both clades of deuterostome and protostome (1). It is essential to understand what kinds of genes had existed in the central nervous system (CNS) of the common ancestor between deuterostome and protostome for the evolutionary study of CNS. To answer this question, we took a comparative approach using different species, particularly focusing on one of the lower bilateral animals, planarian (Platyhelminthes, Tricladida) which is known to possess the CNS. We determined the nucleotide sequence of expressed sequence tags (ESTs) from the head portion of planarians, obtaining 3,101 non-redundant EST clones. As a result of homology search, we found that 116 clones had significant similarity to the genes related to the nervous system. Here, we compared these planarian 116 EST clones with all ORFs of the complete genome sequences of human, fruit fly, nematode, showing that over 95% of these 116 nervous system-related genes were commonly shared among these organisms, suggesting the existing of common ancestral CNS at the molecular level. Interestingly, we found that about 30% of planarian nervous system-related genes had homologous sequences in *Arabidopsis* and yeast which do not possess the nervous system. It implies that the origin of nervous system-related genes was much older than the emergence of the nervous system. A similar approach can be taken for the evolutionary studies of MHC.

**(S3-6) Co-evolution of natural killer cell receptors and MHC class I molecules in higher primates**

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Natural killer (NK) cells are lymphocytes of innate immunity that secrete cytokines and kill infected cells at early times in infection. By interaction with dendritic cells NK cells influence the temporal transition from innate to adaptive immunity. NK cells use several different families of receptors to interact with other cells, including ones with specificity for major histocompatibility complex (MHC) class I molecules and MHC class I-like molecules. In mammals two genetic complexes specify MHC class I receptors of NK cells: the natural killer complex (NKC) that encodes receptors with lectin-like structures and the leukocyte receptor complex (LRC) that encodes receptors made up of immunoglobulin-like domains. The MHC the NKC and the LRC are all situated on different chromosomes. As for the MHC class I and class II genes, certain families of NKC and LRC genes are characterized by diversity, genetic polymorphism and relatively rapid rates of evolution. Consequently, their structures and functions differ quite strikingly between species, as was first appreciated from comparison of human with mouse. Whereas the receptors for polymorphic MHC class I molecules in mice are lectin-like Ly49 molecules

encoded by a polymorphic gene family of the NKC, in humans the functionally analogous receptors are killer cell immunoglobulin-like receptors (KIR) encoded by a polymorphic gene family of the LRC. A current working model is that the species divergence reflects strong pressures upon the NK-cell response to infection and the need to maintain functional interactions with rapidly evolving MHC class I ligands.

Further investigation of these questions requires comparison of species that are more closely related than humans and mice. We have therefore begun to compare the human KIR system with its counterpart in species of ape: chimpanzee, bonobo, gorilla and orangutan. And research by others of rhesus monkey also permits comparison with this Old World monkey species. The human KIR gene family is compact and contains three conserved genes: one at each end and one in the middle (KIR2DL4). The regions between the conserved genes vary in the numbers and the types of KIR genes they contain, being favoured sites for gene duplication, deletion and recombination. There are three phylogenetic lineages of human KIR and all of these are represented in each species of ape. Although gene lineages are conserved in the hominoids only a minority of the individual KIR genes are common to species and only one (KIR2DL4) is common to all. The rhesus monkey KIR are more divergent and most comprise a distinct gene lineage. KIR2DL4 is, however, present. The more rapidly evolving KIR genes appear to be those with specificity for polymorphic MHC class I determinants. In particular the system of HLA-C ligands and HLA-C specific KIR, whose functions appear dominant in humans, is of most recent origin and refinement. In orangutans the MHC-C/KIR system is at an intermediate stage of complexity and in rhesus monkey it is absent.

## Workshop

### **(WS1-2) IL-10-producing alloreactive cells and the lack of development of acute GvHD post bone marrow transplant**

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Using a murine model, Hoffmann (2002), demonstrated rescue of recipients from lethal GvHD using freshly isolated CD4+ CD25+ from unprimed mice. These authors also noted that protection was dependent of IL-10 production. Human CD4+ CD24+ regulatory T cells (Treg) isolated from peripheral blood have been shown to suppress alloresponses in the MLR (Jonuleit 2001, Leving 2001). Jonuleit (2001) observed that contrary to murine regulatory T cells, human Treg, produced high levels of IL-10.

The study group of 26 patients from 6 transplant centres received bone marrow transplants from HLA identical sibling donors as treatment for their haematological disorders over the period January 1990 to June 2000. Peripheral Blood Mononuclear Cells (PBMC) from both recipient and donor taken before transplant were cryopreserved and stored. Patients analysed in this study all received a myeloablative treatment regime and the GvHD prophylactic drug treatment after transplantation was Methotrexate and Cyclosporin. Transplanted marrow was not manipulated prior to grafting.

IL-10 cytokine polymorphisms were identified using PCR-SSP technology. The frequency of IL-10-producing cells was determined using an ELISPOT assay adapted specifically for the purpose of detecting alloantigenic responses.

Analysis of the data presented demonstrated failure to develop acute GvHD after HLA identical sibling bone marrow transplant correlated with an elevated frequency (> 1 per one thousand PBMC) of IL-10-producing T cells present in the donor in response to alloantigen.

These results have significant implications for improving the fate of bone marrow transplant recipients and identify a potential immunotherapeutic for the management of patients post transplant.

### **(WS1-3) LABScreen™ PRA evaluation for HLA class I antibody screening for thai patients awaiting kidney transplant**

Pawinee Kupatawintu<sup>1</sup>, Sirilak Phiancharoen<sup>1</sup>, Araya Tatawathorn<sup>1</sup>, Daowan Wiriyapong<sup>1</sup>, Oytip Nathalang<sup>2</sup>  
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The aim of this study was to compare the detection of HLA class I antibodies by two different techniques, the complement-dependent cytotoxicity (CDC) and the LABScreen™ PRA. The study included 232 serum samples from Thai patients awaiting kidney transplantation. Ninety-three sera (40.09%) were negative and 121 (52.16%) were positive by both techniques. Seven sera (3.02%) were positive only by CDC whereas 11 sera (4.74%) were positive only by LABScreen™ PRA. It was found that LABScreen™ PRA gave a sensitivity of 94.5% while the specificity was 89.4%. Moreover, result discrepancies in 18 serum samples were tested with LABScreen™ PRA. The disagreement results of antibody specificities were due to HLA polymorphism and the different panels used.

It is concluded that LABScreen™ PRA seems to be a sensitive technique for HLA class I PRA testing when compared with CDC. Additionally, since HLA antigen frequencies differ among ethnic groups, the most appropriate panel selection should be considered.

### **(WS1-4) Polymorphism of microsatellite markers in HLA region and effects on clinical outcome of unrelated hematopoietic stem cell transplantation**

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Abstract: Graft-versus-host disease (GVHD) is one of the most important complications in patients who receive HLA-A, -B, -C, and -DR and -DQ matched hematopoietic stem cell transplantation (HSCT) from unrelated donors. The effect of mismatching for microsatellite existed in HLA region on acute GVHD and overall survival is still unknown. In this study, the polymorphism of eight microsatellites in HLA region were investigated and the effect of the mismatching was analyzed on the clinical outcome of the 100 recipients who received complete HLA matched HSCT through Japan Marrow Donor Program. Low degree of mismatches was observed in DQCARI (4%), MICA (7%), MIB (11%), and C1-3-1 (7%), while, high degree of mismatches was observed in TNFd (27%), TNFa (27%), D6S273 (36%)

and C3-2-11 (56%). There was no association between mismatching of these microsatellites and the incidence of GVHD and the recipient survival. The genetic predisposition to GVHD was also investigated in this study by analyzing each microsatellite allele frequency. The frequency of DQCAR II\*200 showed a significant decrease in patients with acute GVHD (grade I–II) in comparison to patients without the same complication ( $P < 0.0008$ ,  $RR = 0.06$ ). This allele may consider as an independent genetic marker for protection against mild acute GVHD. In spite of, TNFa\*113 showed a significant increase in patients with severe GVHD (grade III–IV) in comparison to other recipients. ( $P < 0.0084$ ,  $RR = 3.89$ ). It may reveal as an inductive genetic marker for acute GVHD. However the results of present study showed no strong association between aforementioned microsatellites and GVHD, further study on other microsatellites is necessary for finding others in linkage disequilibrium with minor histocompatibility genes, which are effective candidates for GVHD.

### **(WS1-5) Short term outcome in high risk renal transplant recipients with newer immunosuppressives**

Gurvinder Singh Chopra, P P Varma, S R Gedela, K V Baliga, S K Nema, R Rai  
Army Hospital (Research & Referral)

We studied short term graft outcome in high risk rejection group with newer immunosuppressive drugs. Protocol of our center is cyclosporine, azathioprine and prednisolone. Patients receiving second transplant/having high PRA/receiving unrelated/cadaveric kidneys were classified high risk. They were given plasmapheresis/IL-2 receptor blocking agents and MMF instead of azathioprine. Use of ALG/ATG was restricted to cadaveric kidneys/patients whose PRA remained unresponsive to plasmapheresis/steroid resistant rejection. Over the last 16 months 72 transplants were done, of which two grafts were lost due to graft artery thrombosis. 70 patients formed the study group, of which 25 formed the high risk group(GpA) which included 08 re-transplants, 04 high PRA, 04 with cadaveric grafts and 17 were spousal transplants; balance 45 formed the low risk group(GpB). Mean age of GpA was 35.5 years (18–55) with M:F ratio 17:8. Basic immunosuppressive protocol in these patients was CyA, MMF and prednisolone. Four patients with high PRA were given plasmapheresis; four cadaveric graft recipients were given sequential quadruple therapy with ATG, CyA, MMF and prednisolone. Eight re-transplant recipients received IL-2R blockers in addition. Mean follow-up of these patients was 07 months (1–16). Nine patients (13%) developed acute rejection, (5/25) 20% in GpA and 4/45(9%) in GpB ( $p > 0.05$ ). All rejections could be reversed with steroids except one in each group which required ATG. Mean serum creatinine of GpA and GpB after 07 months follow-up was 1.5 mg/dL and 1.4 mg/dL respectively. The study highlights that with judicious use of newer immunosuppressives, excellent short-term graft survival can be achieved, even in high-risk groups.

### **(WS1-6) Monitoring of cytokine production as a predictor of acute graft rejection in live related donor (LRD) renal transplantation**

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The present study is aimed at monitoring effector cytokine producing helper T cells by intracellular staining for IFN- $\gamma$  and IL-4 at pre transplant and on days 7, 15, 30, and 90 following LRD renal transplantation. The pre transplant frequency of IFN- $\gamma$ (7.55  $\pm$  5.01%, median 6.16) and IL-4 (1.56  $\pm$  1.07% median 1.21) expression was significantly decreased in patients as compared to healthy controls (15.73  $\pm$  10.22, median 13.06, and 1.92  $\pm$  1.73%, median 1.56 respectively,  $p = 0.002$ ). Nine of the fifty four patients who suffered acute rejection episodes had significantly heightened response for IFN- $\gamma$ (19.16  $\pm$  7.4% median 17.50) but not of IL4 (2.69  $\pm$  1.97%, Median 1.8) at the time of acute rejection as compared to changes in their pre Tx levels (5.68  $\pm$  1.63%, Median 5.20) and also with the non-rejectors (5.97  $\pm$  4.39%, median 4.3  $p = 0.0004$ ). However, patients with impaired renal function due to causes other than acute rejection (non-immunological causes of dysfunction) had lower levels of IFN- $\gamma$  than their pre-transplant levels (4.10  $\pm$  2.37%, Median 3.87 vs 9.71  $\pm$  6.02%, Median 7.60) and the differences were significant when compared with that of the acute rejectors ( $p = 0.008$ ). Although the IL-4 expression showed a marginal increase from the pre transplant level (1.15  $\pm$  1.05% vs 0.92  $\pm$  0.47%), this was not significant when compared with that of acute rejectors. our study showed that regular cytokine monitoring in renal transplant patients could provide an effective tool for immunological monitoring of acute rejection and functional assessment of immunosuppression during post transplant follow up.

### **(WS1-7) Natural Killer Receptor KIR genotype and HLA-C KIR epitope incompatibility in acute GVHD of unrelated bone marrow transplantation.**

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Killer Ig-like receptor (KIR) is expressed on NK cells and recognizes HLA class I antigens to regulate cytotoxicity or cytokine secretions. To evaluate the effects of KIR on transplantations, KIR epitope and KIR genotype of recipient-donor pairs from unrelated bone marrow transplantation through Japan Marrow Donor Program (JMDP) have been analyzed. We determined the HLA-C KIR epitope (G1 and G2) compatibility between the recipients and donors. Frequencies of acute GVHD is higher in KIR epitope mismatched cases than in matched cases when the mismatch is GVH direction for the inhibitory receptor. We analyzed genotype of 14 KIR genes of the recipient-donor pairs by the PCR-SSP method. In GVH direction mismatch (recipientG2G2-donorG1G2), all donor examined has the inhibitory KIR gene for G1 and G2. There are no mismatch cases between inhibitory KIR genotype of the donor and corresponding KIR epitope of the recipient, suggesting the selective expression of inhibitory KIR on the cell surface of the donor is responsible for the acute GVHD. On the contrary, high frequency of the acute GVHD was observed when the donor has the activating KIR gene for G2 (KIR2DS2). In the case of HVG direction mismatch (recipientG1G2-donorG2G2), GVHD frequency is not high even when the donor has the KIR2DS2. These results indicated that activating KIR genotype and the corresponding KIR epitope incompatibility has effects on the acute GVHD. Data also suggested the involvement of NK cells in GVHD and the importance of the KIR genotype and KIR epitope matching in unrelated bone marrow transplantations.

### **(WS2-1) Abundant heterogeneity of HLA expression and aneuploidy in head and neck squamous cell carcinomas.**

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HLA has a pivotal function in the immune system. Presentation of endogenous derived peptides by HLA class I and B2m offers the immune system a mechanism to monitor intracellular events. Infection by bacteria or viruses and tumorigenesis alters the peptide pool presented on the cell surface. This may lead to a cytotoxic T-cell response by which non-healthy cells can be eradicated. In tumors, loss of HLA cell surface expression is frequently observed. The reduced HLA expression affects the possibility to detect non-healthy cells (e.g. cancer cells) and is therefore a mechanisms by which tumor cells can circumvent eradication by cytotoxic T-cells. In tumors, HLA and B2m gene loss is frequently studied to characterize HLA loss phenotypes. Microsatellite markers are widely used to study loss of heterozygosity (LOH). We studied LOH of the B2m gene in 53 head and neck squamous cell carcinoma (HNSCC) using an expanded set of microsatellite markers compared to described in literature and combined the results with immunohistochemical staining of B2m. Four HNSCC had LOH and showed positive or overexpression of B2m. Since LOH can not distinguish loss from amplification we determined the copy number of chromosome 15 (where B2m is located) using centromere 15 fluorescent in situ hybridization (FISH) in 11 HNSCC including the 4 with LOH and positive B2m expression. Centromeric chromosome 1 FISH was used as control. Summarized, chromosome 15 as well as chromosome 1 was affected in all 11 HNSCC. The majority of the tumors were heterogeneous for the chromosome 15 and 1 copy number. Amongst others, the four HNSCC with LOH and B2m expression showed amplification of chromosome 15 instead of loss. It can be concluded that B2m microsatellite LOH does not reflect the B2m gene copy number in these HNSCC. Moreover, LOH does not only detect loss but also amplification of a genomic region. Preliminary results of ongoing studies on LOH of chromosome 6 in relation to HLA expression and aneuploidy display similar characteristics as described for B2m.

#### References:

- Koene G, Van der Ven K, Verdaasdonk M, Slootweg P, De Weger R, Tilanus M. A variety of HLA phenotypes in HNSCC patients identified with the workshop antibodies as defined in the HLA and Cancer component. IHWG 13th workshop proceedings, in press.
- Koene G, Coenen S, Verdaasdonk M, De Weger R, Slootweg P, Tilanus M. The value of loss of heterozygosity of STR markers to define expression variants of the B2M gene. IHWG 13th workshop proceedings, in press.
- Koene G, Arts Y, Van der Ven K, Rozemuller E, Slootweg P, De Weger R, Tilanus M. Fluorescent in situ hybridization and loss of heterozygosity B2-microglobulin in Head and Neck Squamous Cell Carcinomas to define the B2 microglobulin gene copy number. Submitted for publication.

### **(WS2-3) Pathogenicity and the involvement of HLA-B27 subtypes in Spondyloarthropathy of North Eastern Indian population**

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Spondyloarthropathy represent a complex heterogenous group of genetic disorders that share a number of clinical, radiographic, genetic features and are quite distinct from rheumatoid arthritis. HLA-B27 is a serologic specificity that encompasses 25 different alleles that encode 23 different product (HLA-B\*2701 to HLA-B\*2723) which are associated with diseases. Ankylosing spondylitis and HLA-B27 antigenic positivity, is one of the strongest association seen between a disease and a Class-I antigen.

The genetic subtypes and susceptibility to development of disease vary in different ethnic population which may be result of genetic and geographic origins. In present investigation unrelated patients with spondyloarthropathy (Indian-born Bengali population = 52) has been considered to investigate which of them are and are not associated with AS and related SpA, and whether certain subtypes show any preferential association with some of the clinical features or forms of these diseases among the said population.

Serological typing was performed in all the 52 cases after assessing several clinical criteria like extra articular features, radiological findings, determination of RF, ANA, ENA, ESR, CBC, C-reactive protein, arthroscopy etc.. PCR based high resolution molecular typing was conducted on 19 serologically HLA-B27 positive patients, 8 of them showed the presence of B27 gene. From molecular typing results, we observed a significant preferential association (75%) of B\*2752/53 gene in this population. Significant findings have also arisen from the study: B\*2704, B\*2705, B\*2708.

The presence of HLA-B27 gene may serve as an aid to diagnosis or prognosis for clinicians though how it contributes disease, its role remains enigmatic.

### **(WS2-4) Etiologic considerations of major histocompatibility linked genes in paranoid psychosis: a possible relationship between delusional disorders and paranoid schizophrenia.**

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Paranoid symptoms are among the most dramatic and serious disturbances in psychiatry and medicine. Delusional disorder, the contemporary conceptualization of paranoia, is an uncommon condition characterized by the presence of one or more nonbizarre delusions and the relative absence of associated psychopathology. Paranoid (delusional) disorders are usually thought to overlap with schizophrenia disorders, which is characterized by the presence of prominent delusions or auditory hallucinations and perceptual disturbances. Delusional disorder is probably heterogeneous group of illness and occurs in a variety of psychiatric and medical conditions. Etiology of delusional disorder is poorly understood. Recently the involvement of genetic factors and familial aggregation of the disease have been suspected. The main purpose of the present study was to investigate the role of HLA Class-I antigens in the patients with delusional disorder and Paranoid Schizophrenia. A total number of 80 unrelated patients with delusional disorder and 30 unrelated patients with paranoid schizophrenia along with 80 healthy individuals considered for the study were selected from the Indian born Bengali population. The frequency of HLA Class-I genes have been studied by PCR-SSP typing. Our results suggest a strong association of HLA-A3 antigen (60% vs. 15%) with delusional disorder and paranoid schizophrenia. (53.33% vs. 15%). From the result it can be interpreted that HLA-linked genes may have the role to increase susceptibility to paranoid disorders. Also delusional disorder may have a causal link with paranoid schizophrenia and/or delusional disorder may be one of the possible sources of the heterogeneity within "schizophrenia".

### **(WS2-5) Comparison of HLA-B51 haplotypes and microsatellite polymorphisms in Bechet's disease of multiple populations**

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Bechet's disease (BD) is a chronic multifactorial disorder, with genetic and environmental factors. The distribution of BD is mainly around the Mediterranean Sea, Middle East and Far East. Previous studies have shown that BD has an primary association with HLA-B\*51011 in many different ethnic groups, suggesting that HLAB\*51011 determines the susceptibility to BD as one of major risk factors. However, HLA class I haplotypes are composed of not only the HLA-B gene but also of many functional and unfunctional genes which are located in the HLA class I region. Recent studies showed that microsatellite markers, such as MICA-TM, MIB and C1-4-1, have significant associations with BD. The aim of this study was to clarify the association of HLA haplotypes and microsatellite polymorphisms, and to make phylogenetic analysis on HLA-51 by making a comparison of HLA haplotypes and microsatellite polymorphisms in Turkish, Iranian and Japanese Bechet's patients to trace an ethnic origin of BD. HLA-A and -B haplotype analysis, investigation of microsatellite markers dispersed throughout the HLA class I region and direct sequencing of the HLA-B gene were performed. As a result, unique linkage among HLA haplotypes, microsatellite polymorphisms around HLA-B and single nucleotide polymorphisms (SNPs) of HLA-B could be found in BD.

### **(WS2-7) Human leukocyte antigens class I and II, In Iranian patients with common variable immunodeficiency**

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Background: Common variable immunodeficiency (CVID) is a heterogeneous group of primary immunodeficiency disease characterized by hypo gamma-globulinemia and variable or low numbers of circulating B cells. An association between CVID and abnormally high frequency of an HLA haplotype including HLA-A1, HLA-B8, HLADR3 (referred to as haplotype 1) and another one including HLA-A29, HLA-B44, HLA-DR7 (Referred to as haplotype 2) has been previously documented. In the present study, we have attempted to find an association between susceptibility to CVID and HLA Class I and Class II antigens in Iranian population. Subjects and method: 20 Iranian patients with CVID (mean age 17, range 3–28 years; 12 males and 8 females), and 100 healthy controls were studied. we used PCR based HLA typing for Class II and micro-lymphocytotoxicity technique for Class I typing. results: Out of 20 CVID subjects typed for HLA-DR, DQA1 and DQB1 specificities, the most frequent alleles in the patients population were DRB1\*1303 (8.3% vs 0.5%, od = 18.09, Pc = 0.01), HLA-DRB1\*04 (16.6% vs 10.5%), DQB\*10302 (12.9%vs 5.5%), DQB\*1020 (25.8% VS19%) and DQA1\*0500 (49.9% VS 39%). The least frequents alleles in the patients population were HLADRB1\*15 (5.5% VS12 ) AND dqb1\*05011 (6,4% vs 22% od = 0.24, P = 0.07). We could not find any significant association between HLA-Class I previously reported to be associated with CVID patients.' Conclusion: Our results suggest that alleles in the HLA-DRB, DQB1 and DQA1 loci may contribute to susceptibility to Iranian patients with CVID.

### **(WS2-8) Genes influencing innate and acquired immunity in type 1 diabetes**

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T1DM is very common in Sweden and is positively associated with HLA class II genes. Approximately 89% of the newly diagnosed patients carry the high-risk HLA-DR4-DQ8 and DR3-DQ2. The remaining 11% develop T1DM without them. This can be due to involvement of other genes and environmental factors. NK cells of the innate immune system are important in anti-viral and anti-tumor immunity. They are implicated in the etiology of autoimmune T1DM. Human NK cells express Killer Immunoglobulin-like receptors (KIR) which belong to polymorphic multigene family in chromosome 19q3.4. They modulate NK cell response by interacting with HLA class I. In addition polymorphic MICA in HLA class I interacts with non-polymorphic NKG2D receptor on NK cells. We have studied, in addition to HLA-DR and DQ, genes of the innate immune system MICA and KIR in Latvian patients (n = 98) and controls (n = 100) with T1DM. They were genotyped using standard PCR based typing methods. MICA allele 5 to be positively associated with T1DM. KIR2DL2 and KIR2DS2 were both positively associated. Combined association of MICA5 and KIR2DL2 gave an odds ratio (OR) of 26.7. However

the combined risk of KIR2DL2 and HLA class II genes, HLA-DR3 (OR = 73.4), DR4 (OR = 66.8) and DR3 and DR4 (OR = 88.3), were higher. The maximum risk was when KIR2DL2, MICA5 and DR3/DR4 were in combination.

In conclusion, our results suggest that a balance between innate and acquired immunity is important and an imbalance could lead to T1DM.

### (WS2-9) Molecular basis of HLA and disease susceptibility to tuberculosis

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Structural and functional correlation of MHC-peptide interaction has led to newer understanding of the role of susceptibility gene(s) in diseases. To demonstrate the susceptible/resistant peptide-binding motif in patients with pulmonary (both drug sensitive and resistant) and extra-pulmonary TB, we analyzed HLA class I specificities based on the supertype classification and amino acid variations of class II peptide-binding pockets '1' & '4'. Of the nine HLA-A and-B superotypes, A3-like and A1-like superotypes were inversely associated in patients with pulmonary as well as miliary/disseminated TB. These two disease associated superotypes share similar peptide binding motif except 'Pocket F' of the HLA class I molecules. In addition, the HLA-Cw specificities that are major ligands for killer cell Ig-like receptors (both KIR2DL1, DL2) were encountered more frequently among TB patients suggesting a possible inhibition of NK cell activity against the infected target cells. Similarly, sequence analysis of HLA-DRB1 alleles particularly in the 'pocket 4' that have a dual role of peptide binding as well as T cell recognition, showed positive association of negatively charged (D<sup>70</sup>E<sup>71</sup>A<sup>74</sup>) and neutral charged (Q<sup>70</sup>A<sup>71</sup>A<sup>74</sup>) 'pocket 4' motif with tuberculosis. Further, simultaneous evaluation of 'pocket 4' and V/G dimorphism at position  $\beta$ 86 of 'pocket 1' revealed significant over representation of valine bearing alleles among patients with extensive lung lesions than those with minimal disease. These results underline the importance of specific peptide-binding pockets in the MHC molecules and ligand specificity of the KIR receptors in governing susceptibility and disease severity following M. tuberculosis infection.

### (WS2-10) Variations in cytokine genes influence mycobacterial disease profiles

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Dept Transplant Immunol & Immunogenet. All India Institute of Medical Sciences

With the recent corollary of knowledge gained on Th1/Th2 paradigm, cytokine gene polymorphism, interindividual differences have been discovered that influence not only the gene expression but also susceptibility to and clinical severity of disease. This study has evaluated polymorphism in cytokine genes (IL1a, IL1b, IL1R, IL1RA, IL4RA, IL12, gIFN, TGFb, TNFa, IL2, IL4, IL6 and IL10) using PCR-SSP technique (developed by Dr J Mytilineos) among tuberculosis patients from India, Greece (collected by Dr P Pratsidou-Gertsis) and leprosy patients from India and Korea (collected by Dr Gue Tae Chae). Significant observations included increased frequency of (i) IL 4 haplotype TCC in Indian LL, (ii) IL1RA mspa 111100 C in Indian TL, (iii) IFN $\gamma$ UTR 5644 AA in Indian TL (iv) IFN $\gamma$ UTR 5644 A, IL4-1098 G, IL2-330 TT, and IL10-819 CT in Indian TB, (v) IFN $\gamma$ UTR 5644 T, TGFb1 CG and TGF $\beta$ 1 cdn 10 CC in Greek TB, (vi) IL-511 C, IL 4-33 C and IL4-33 CT among the Korean TL, (vii) IL4-33 C also in Korean LL, (viii) IL1 $\beta$ -511 CC, IL1RA mspa 111100 CC, IL4-590 CT in Korean TL, and (ix) IL6-174 CG amongst Korean LL patients. Likewise associations with decreased frequencies of (i) IL4 GCC in Indian TL, (ii) IL1RA mspa 111100 C in Indian LL, (iii) IL1RA mspa 111100 CT, IL4-33 TT in Korean TL, (iv) IL4-33 CT in Korean TL, (v) TGF  $\beta$ 1 cdn 10 CT and IFN $\gamma$ UTR 5644 AA in Greek TB were also observed.

### (WS3-3) Allelic and haplotypic diversity of HLA-A, -B, -Cw, -DRB1, and -DQB1 genes in the Korean population

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Development of high level of HLA typing technology exposes the unique pattern of HLA allele and haplotype frequencies in each population. In this study, genotypes of HLA-A, -B, -Cw, -DRB1, -DQA1 and -DQB1 loci were analyzed in 485 apparently unrelated healthy individuals of Koreans. A total of 20 HLA-A, 43 HLA-B, 21 HLA-C, 31 HLA-DRB1, and 14 HLA-DQB1 alleles were identified. Eleven alleles (A\*0201, A\*1101, A\*2402, A\*3303, B\*1501, Cw\*0102, Cw\*0302, Cw\*0303, DQB1\*0301, DQB1\*0302, DQB1\*03032) appeared to be major alleles exhibiting more than 10% in the population. In each serologic group, less than 3 alleles exhibited only with several exceptions (A2, B62, DR4, DR14, DQ6). Multiple locus haplotypes estimated by maximum likelihood method revealed 51 A-C, 43 C-B, 52 B-DRB1, 34 DRB1-DQB1, 48 A-C-B, 42 C-B-DRB1, 46 B-DRB1-DQB1, and 30 A-C-B-DRB1-DQB1 haplotypes with the frequencies of more than 0.5%. Eighteen of DRB1-DQB1 and 16 of B-C haplotypes exhibit tight associations (RLD > 0.95). The five

locus haplotypes defined by high level of DNA typing correlated well with previously identified serology-based haplotypes in the population. The five most frequent haplotypes are: A\*3303-C\*1403-B\*4403-DRB1\*1302-DQB1\*0604 (4.2%), A\*3303-C\*0701-B\*4403-DRB1\*0701-DQB1\*02 (3.0%), A\*3303-C\*0302-B\*5801-DRB1\*1302-DQB1\*0609 (3.0%), A\*2402-C\*0702-B\*0702-DRB1\*0101-DQB1\*0501 (2.9%), and A\*3001-C\*0602-B\*1302-DRB1\*0701-DQB1\*02 (2.7%). Several sets of allele level 5-locus haplotypes that has impact on unrelated hematopoietic stem cell transplantation but could not be discriminated by routine HLA-A, B, DR low level typing, are mostly originated from allelic diversity of A2, A26, B61, and DR4 serologic groups. Information obtained in this study will be useful in medical and forensic area as well as in anthropology in the population.

#### **(WS3-4) Genomic diversity of HLA in North Indians characterizes higher combinatorial variety than allelic diversity.**

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The North Indian population displays extensive diversity alongwith novel alleles and unique haplotypic combinations in its HLA repertoire. Allelic data indicate an important role of microbial pressure and possible oriental influence in generation of these novel alleles and in shaping this repertoire. Extensive heterogeneity in the haplotype repertoire is indicated by the statistic that 60% of HLA-A-B haplotypes are observed only once in the population while only 13% are represented more than 4 times. Similarly 41% of HLA-DRB1-DQA1-DQB1 haplotypes occur only once in the population while 30% are represented more than 4 times in the cohort studied. Highest linkage disequilibrium is observed for the ancestral haplotype AH8.2, HLA-A\*26-B\*08-DRB1\*03 (%HF = 5.1), which is unique to the Indian population. In addition to representation of typical Caucasians and Oriental haplotypes, a large number of unique haplotypes, particularly in the DR2 family were also encountered in this population.

On the other hand, the allelic repertoire revealed comparatively lower heterogeneity with 7–14 alleles, at each of the classical class I and II loci, making up more than 50% of the allelic conjugate of the population with an average homozygosity of 11%–30%. The HLA-C locus however revealed much higher homozygosity (49%) with only 4 alleles collectively constituting greater than 50% phenotypes in the population. These data indicate a much higher combinatorial heterogeneity in the North Indians compared to the allelic diversity, possibly due to extensive recombinations owing to genetic admixture and subsequent selection and may have important implications on immunobiology and immunotherapy trials in this population.

#### **(WS3-5) MICA polymorphisms in Pacific Islands populations**

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Australian Red Cross Blood Service

The MICA gene is a homologue of the MHC class I genes located approximately 40 kb centromeric to the HLA-B locus. The gene encodes cell surface molecules with a1, a2, and a3 extracellular domains and a transmembrane domain. The expression of MICA gene is largely confined to epithelial cells. MICA peptides are ligands of the NKG2D receptor expressed on NK cells, CD8+ ab T cells and gd T cells, and as such are part of an innate immune response. It has been shown that the MICA gene is highly polymorphic with more than 50 alleles identified to date. Similar to HLA genes, allele frequency distribution of the MICA gene varies between different ethnic groups. We have identified MICA allele frequencies in four Polynesian populations: Cook Islands, Samoa, Tokelau and Tonga using sequencing based typing. We have found a limited diversity of MICA alleles in Polynesians with a few predominant alleles. Correlation with allele frequency distribution in other populations and MICA-HLA haplotypic associations are discussed.

#### **(WS3-6) Sequence analysis of the ATP6G and NFKBIL1 genes in four HLA haplotypes commonly seen in the Ami tribe.**

Chen-Chung Chu, Zong-Xian Chen, Jean Alain Trejaut, Marie Lin  
Mackay Memorial Hospital

The Ami tribe, one of ten indigenous tribes in Taiwan, has very high frequencies of HLA-DRB1\*0404 and 0405 (gf = 0.35 and 0.21, respectively). Although these two alleles may be associated with the development of rheumatoid arthritis (RA, RA is rarely seen among the Ami tribe. It is believed that others genetic and environmental factors may contribute to the disease development. Recently, a second susceptibility gene associated to rheumatoid arthritis was suggested to be present in a 70 kb segment, telomeric to the TNF gene region in the HLA class III region, which including NFKBIL, ATP6G, BAT1 and MICB genes. In this study, we propose to survey the genetic polymorphism of the ATP6G and NFKBIL1 genes.

Direct sequencing of long-range PCR products were performed in four HLA-A-B-DRB1 homozygous individuals with the following

haplotypes: A\*2402-B\*4001-DRB1\*0404, A\*2402-B\*4001-DRB1\*0405, A\*3401-B\*5601-DRB1\*1502 and A\*2402-B\*4801-DRB1\*0404. Four long-range PCR products were amplified from each individual and were sequenced in both directions using 166 sequencing primers.

In this report two long-range PCR products including all predicted exon regions of ATP6G and NFKBIL1 genes were sequenced and compared to the NCBI reference sequence. There was no sign of heterozygosity, and among a 3 bp deletion, a 4 bp insertion, two 1bp insertion and 10, 9, 15 and 8 substitutions seen respectively in the four individuals, only one synonymous substitution (C31588548T) belonged to the coding region. C31588548T was shared by haplotypes B\*5601-DRB1\*1502 and B\*4801-DRB1\*0404. Further analysis are still being performed, and any new outcomes will be reported.

### **(WS3-7) Microsatellites in the HLA region, Association with HLA alleles and HLA haplotypes**

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More than 250 highly polymorphic Microsatellites markers exist in the HLA region. Some of them are known to be in linkage disequilibrium (LD) with HLA. Their study allow to refine the analysis of HLA-region LD structure which is a very important issue in the selection of appropriate markers to use in studies of MHC molecular genetics and disease association.

Population and Methods: We present here data on 2183 unrelated haematopoietic stem cell donors already typed for HLA-A HLA-B and HLA-DR and typed for 16 Microsatellites. Identification of HLA association (Pair wise linkage disequilibrium D, Sensitivity Se, Specificity Sp, Odds Ratio, Hellinger measure) with Microsatellites has been studied using haplotype frequency estimation by maximum likelihood implemented in Expectation Maximisation algorithm.

Results: Best predictions have Se values around 80%, Sp around 99% an standardised measure of D 0,8. It allows the identification of multi point association between HLA and Microsatellites For example : \*29-44-07 and TNFd-Mib-C244-D6S265-MOGc-D6S2222; \*02-44-04 and DQIV-DQcar-D6S273-TNFa; \*03-07-15 and TNFd-Mib-C244-D6S265-MOGc; \*03-35-01 and DQIV-Dqcar-D6S273-TNFa-TNFd-MIB-C244-D6S265-MOGc; \*23-44-07 and TNFa-TNFd-MIB-C244-D6S265-MOGc; \*01-57-07 and MIB-C244-D6S265-MOGc; \*02-60-13 and D6S273-TNFa-TNFd-MIB-C244; \*30-13-07 and TNFd-Mib-C244-D6S265-MOGc

Discussion: Such results are only preliminary results. Analysis on 6000 thousands individuals would give more accurate association and would allow better identification of rarer HLA alleles and/or haplotypes with Microsatellites.

### **(WS3-8) Analysis of sequence variations of the MHC class II gene in genus spenicus.**

Eri F Kikkawa<sup>1</sup>, Taeko K Naruse<sup>1</sup>, Michio Fukuda<sup>2</sup>, Masanori Kurita<sup>3</sup>, Koichi Murata<sup>4</sup>, Rory P Wilson<sup>5</sup>, Yvon LeMaho<sup>6</sup>, Michio Tsuda<sup>1</sup>, Tomi T Tsuda<sup>7</sup>, Hidetoshi Inoko<sup>1</sup>

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Penguins known to be branched from diving oceanic birds about 47MYA ago have been classified into six genera which consist of 16 species at present. In the present study, we have cloned and analyzed the MHC class II DRB1 (like) gene polymorphism of Humboldt penguins (*S.humboldti*) as the first step to identify generic variations in the penguin species and to estimate systematically the evolution and specialization of Birds in the context of penguin polymorphism.

Genomic DNAs of 20 wild or captive Humboldt penguins were amplified with a set of primers designed for PCR amplification of a 1.1 kb PCR products containing the exon 2, intron 2 and exon 3 segments of the DRB1 gene Several subclones carrying PCR products were determined for their nucleotide sequences. The thus obtained sequences in exon 2 had at least 5 different alleles with 14 polymorphic sites in the amino acid sequences and 26 polymorphic sites in the nucleotide sequences. Homology search showed 85% identity with DRB1 of *Gallinago medio* and 90% with that of *Gallus gallus* in exon 2, 91% with that *Anas platyrhynchos* and 88% with that *Gallus gallus* in exon 3. However, the intron 2 sequence appeared to be specific for penguin because low nucleotide identity could be observed in comparison among other birds.

Interestingly, unique nucleotide sequences specific for Humboldt penguins could be found, suggesting a possibility that each genera or species of penguins has acquired each specific sequence in the process of their evolution.

## Best Abstracts

### (Best Abstract Best-1) **Effect of Nef-mediated HLA class I down-regulation on ability of HIV-1-specific CD8+ T cells to suppress HIV-1 replication**

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HIV-1-specific cytotoxic T lymphocytes (CTLs) are induced in acute and chronic phases of HIV-1 infection. However, CTLs cannot completely eliminate HIV-1 virus, and HIV-1-seropositive individuals develop AIDS if they are not treated with anti-retrovirus therapy. HIV-1 Nef-mediated down-regulation of HLA-class I on the surface of HIV-1-infected cells is one of mechanisms that allow HIV-1 to evade CTLs. Previous studies showed that HIV-1-infected cells are hardly killed by HIV-1-specific CTLs due to Nef-mediated down-regulation of HLA-class I. In the present study, we investigated the ability of HLA-A\*3303 CTL clones to inhibit HIV-1 replication by using a Nef-positive HIV-1 strain (NL-432) and a Nef-mutant HIV-1 strain (NL-M20A) that expresses a Nef protein which does not induce down-regulation of HLA class I molecules but is otherwise functional. Primary CD4+ T cells were incubated with the HIV-1 strains and co-cultured with five HIV-1-specific HLA-A\*3303 CTL clones. The ability of HLA-A\*3303 CTL clones to inhibit HIV-1 replication was investigated by measuring p24 expression in CD4+ cells by flow cytometry and p24 proteins in culture supernatant by ELISA. The results showed HIV-1-specific CTL clones suppressed replication of NL-M20A (about 70% suppression), while these CTL clones could partially suppress replication of NL-432 (10–40% suppression). In contrast, they failed to suppress the replication of both viruses in HLA-mismatched cells infected with the viruses. These results suggest that HIV-1-specific CTLs partially recognize HIV-1 epitopes presented by HLA-A\*3303 nevertheless the surface expression of HLA class I molecules are down-regulated by Nef.

### (Best Abstract Best-2) **Identification of HIV-1 CD8 T cell epitopes presented by HLA-A\*2601**

Manami Satoh<sup>1</sup>, Yuji Takamiya<sup>1</sup>, Shinichi Oka<sup>2</sup>, Katsushi Tokunaga<sup>3</sup>, Masafumi Takiguchi<sup>1</sup>

<sup>1</sup>Kumamoto University Center for AIDS research, <sup>2</sup>AIDS Clinical Center, International Medical Center, <sup>3</sup>Department of Human Genetics

Since HLA-A\*26 is one of the most common alleles in Asia where approximately 20% people have this allele, identification of HIV-1 CD8 T cell epitopes presented by HLA-A\*26 is necessary for studies of immunopathogenesis in AIDS and vaccine development in Asian. We therefore tried to identify HIV-1 CD8 T cell epitopes presented by HLA-A\*2601. We employed reverse immunogenetics to identify the HIV-1 epitopes presented by HLA-A\*2601. We selected 124 peptides that consist of 8-mer to 11-mer carrying V, T, I, F or L at P2 and Y or F at the C-terminus from HIV-1 SF2 sequence, and then synthesized these peptides. The ability of the peptides to bind the HLA-A\*2601 molecules was tested by a HLA-A\*2601 stabilization assay using RMA-S-A\*2601 cells. 27 peptides bound to HLA-A\*2601. The ability of these HLA-A\*2601-binding peptides to induce peptide-specific CD8 T cells was tested by stimulating PBL from HIV-1-infected individuals having HLA-A\*2601. Stimulated PBL was cultured for approximately 2 weeks and then the ability of the cultured cells to produce IFN- $\gamma$  was tested by stimulation with C1R-A\*2601 cells pulsed with the peptides. 5 of 27 HLA-A\*2601-binding peptides induced peptide-specific CD8 T cells. Of CD8 T cells specific for 5 peptides, those specific for 3 peptides showed IFN- $\gamma$  production after stimulation with C1R-A\*2601 cells infected with HIV-1 recombinant vaccinia virus. These results indicate that these three peptides are HIV-1 CD8 T cell epitopes presented by HLA-A\*2601. This study will contribute to studies of AIDS immunopathogenesis and vaccine development.

### (Best Abstract Best-3) **Expression of non-classical MHC class I molecules in the neuronal cells of central nervous system**

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In order to elucidate a role of MHC molecules in the central nervous system (CNS), we have examined the expression of classical (Ia) and non-classical (Ib) class I MHC molecules in the CNS of C57BL/6 mice by using RT-PCR and immuno-histochemistry. In Ia molecules, although *H2-K* and *H2-D* transcripts were found by RT-PCR analysis, these proteins were mainly localized in endothelial cells and choroid plexus cells by immuno-histochemical study using an anti-Ia antibody. The staining of neuronal cells were faint or weak, and the special distribution of Ia proteins were not recognized in neuronal cells. In contrast, Ib proteins were localized mainly in neuronal cells. The strong

staining of a Qa-1 protein was observed in the nerve cells localized in the 5 and 6th layers of cerebral cortex, hippocampus and the tuberal nucleus. The immuno-staining with an anti-TL antibody revealed that the majority of the nerve cells in cerebrum were positively stained, and the strong staining was observed especially on the nerve cells in the paraventricular nucleus thalamus, and Purkinje's cells in cerebellum. However, *TL* mRNA encoded by *T3* or *T18* gene, was not found. Among the *T*-locus genes, *Qa-1* and *T22* transcripts were confirmed by sequence analysis of RT-PCR products. The specific localization of Qa-1 and some Ib molecules cross reacted with an anti-TL antibody, may suggest an important role of Ib molecules in the remodeling and plasticity of connections of nerve cells or in some immune response in the CNS.

#### **(Best Abstract Best-4) Detection of HLA-F expression in normal and tumor tissues — Collaboration of HLA-E, -F and -G —**

Noriko Sageshima<sup>1</sup>, Ni Lee<sup>2</sup>, Takanori Shobu<sup>1</sup>, Akinori Miki<sup>2</sup>, Katsunori Yane<sup>3</sup>, Saiho Ko<sup>4</sup>, Hideaki Kondo<sup>5</sup>, Motoko Omura<sup>1</sup>, Katsuhiko Hatake<sup>1</sup>, Daniel Edward Geraghty<sup>2</sup>, Akiko Ishitani<sup>1</sup>

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We have investigated the expression of HLA-E, -F and -G to understand their function using monoclonal antibodies we had developed. HLA-G expression is restricted in placenta, which is fetal-maternal border. HLA-E shows ubiquitous expression including placenta. But the expression of HLA-F has not clarified yet. We investigated the surface expression of HLA-F on many kinds of cell lines by FACS and many kinds of normal tissues by immunohistochemical staining and failed to detect the expression in all of them. But when we tested maternal decidua tissue, surprisingly the extravillous trophoblasts deeply invaded into the maternal decidua, expressed HLA-F strongly on the cell surface. On the extravillous trophoblasts, all three nonclassical HLA class I antigens are expressed. This may suggest that they function with associating each other during pregnancy. To understand HLA-F function, we investigated the expression of HLA-F on the human tumor tissues including the expression of HLA-E and -G.

We investigated the HLA-F expression of tumor tissues and leukemic cells by immunohistochemical staining or FACScan, respectively. Although most of tumor tissues and leukemic cells tested showed no expression of HLA-G and HLA-F, one of advanced carcinoma and some of leukemic cells showed HLA-F expression but not HLA-G expression.

It has been thought that HLA-F protein can express on the cell surface. Here, we showed the surface expression of the HLA-F protein on the leukemic cells and tumor. It is suggested that HLA-F may have specific functions and work with HLA-E and -G for fetal-maternal immunoreaction.

#### **(Best Abstract Best-5) EVOLUTION OF THE KIR REGION IN THE GREAT APES**

Lisbeth Ann Guethlein, Laurent Abi-Rached, Laura Flodin, Peter Parham  
Stanford University

The killer cell immunoglobulin-like receptors (KIRs) comprise a highly polymorphic, rapidly evolving gene family. To aid in understanding the evolutionary dynamics shaping this family, we have studied the genomic organization of the KIR region in chimpanzee and orangutan. Phylogenetic analysis of expressed KIR genes in these and other primate species has shown conservation of all major KIR lineages although the number and nature of KIR within each lineage varies.

Analysis of two haplotypes each from chimpanzee and orangutan has revealed conservation of the flanking genes LILR (leukoctye immunoglobulin-like receptor) and FCAR (Fc alpha receptor) in both species. Additionally, all haplotypes correspond to the centromeric half of the human KIR region, suggesting that this is the ancestral organization of the region. The haplotypes from both species include orthologues for two of the three framework genes described in man. The first, KIR3DL3 (KIRCI), located adjacent to the LILR region, is similarly positioned in the orangutan and chimpanzee haplotypes. However, in both chimpanzee haplotypes studied, there is another KIR, Pt-KIR2DL5, located between the Pt-LILRs and Pt-KIRCI. The other framework gene, KIR2DL4, is found in the center of the human KIR region. In both chimpanzee and orangutan, it is the penultimate gene in the complex with a KIR3DL gene located between it and the FCAR gene. Knowledge of genomic structure, discovery of pseudogene sequence, verification of intron/exon structure, and determination of intron sequence have provided invaluable information for the phylogenetic reconstruction of the evolutionary history of the KIR region in the great apes.

## Oral Presentation

### (O1-1) Clinical significance of comprehensive antibody screening in live related donor renal transplantation

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The objective of this study is to evaluate the antibody repertoire against donor T and B cells and monocytes in the recipients of live related donor renal transplantation. Retrospective flowcytometry cross match was done in 81 pretransplant and 90 post transplant sera to evaluate the presence of antibodies against these cells. Nine of the 81 patients tested IgG positive against T cells experienced higher acute rejection episodes at 1 year in comparison to the 72 IgG negative patients (89% vs 38%,  $p < 0.0001$ ). In the antibody negative group, 14 patients lost grafts, 9 (64%) of which were found to have antiendothelial cell antibodies instead. In the remaining 58 patients with functioning grafts, only 12 (21%) developed these antibodies ( $p < 0.0001$ ). During post transplant screening, presence of IgG had a similar impact in both T and B cell positive vs negative groups with a graft survival of 50% vs 92% ( $p < 0.00001$ ) and 72% vs 91% ( $p < 0.05$ ) respectively with higher incidence of acute rejection ( $p < 0.0001$ ) in the positive group. On the other hand, patients elaborating IgA antibodies irrespective of their target cell specificity and IgM (against monocytes) had a better graft survival and lower acute rejection episodes relative to their negative counterparts ( $P < 0.05$ ). These results indicate that an assessment of anti endothelial antibody alongwith comprehensive antidonor antibody screening preferably by flowcytometry provides a more sensitive measure of the sensitization status of the recipient and can prove to be of immense prognostic value in renal transplantation.

### (O1-2) The evaluation of panel reactive antibody assay (PRA) in living renal transplantation.

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(Aim) Many institutions around the world use the flow cytometric panel reactive antibody (FlowPRA) assay to evaluate donor-nonspecific high responders. We have been using the FlowPRA assay at our institution since last year. In this study, we compared the FCXM assay and the FlowPRA assay. (Materials and Methods) A total of 249 samples were retrospectively analyzed using the FlowPRA assay. In 126 out of 249 patients, we compared the results of the FCXM assay and the FlowPRA assay. (Results) 1) Out of 249 Patients, class1 Ab was detected in 40 patients (16%) and class2 Ab in 9 patients (4%), both class1 Ab and class2 Ab in 8 Patients (3%). The rate of class1 Ab and class2 Ab appearance was not related to the number of blood transfusions or pregnancy. 2) In 21 patients who received the first renal graft with FlowPRA (+) and FCXM (-), nine patients (43%) experienced moderate to severe humoral rejection within two weeks of their renal transplantation. 3) Two patients with FCXM (-) and FlowPRA (-) experienced vigorous humoral rejection leading to anuria. (Conclusion) Overall, 43% of the patients who tested FlowPRA (+) at the time of the first renal graft experienced an acute humoral rejection immediately after transplantation, even if the FCXM assay could not detect presensitization. FlowPRA monitoring may be more useful than FCXM monitoring for the detection of high responders. More sensitive monitoring to detect donor-specific antibody, such as single antigen beads FlowPRA, will be further needed.

### (O1-3) Non-T-cell depleted HLA haploidentical stem cell transplantation in advanced hematological malignancies based on the feto-maternal immunological tolerance

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Feto-maternal microchimerism suggests that immunological tolerance exists between mother and fetus. Based on this hypothesis, we performed haploidentical stem cell transplantation (SCT) without T-cell depletion (TCD) in nine patients with advanced hematological malignancies. There were six males and three females, their ages ranging from 17 to 55 years. Two patients had CML at blast crisis, three had ALL/LBL, two had NHL, and two had MM/PCL. All patients were in relapse or refractory stages. HLA incompatibilities for GVHD direction included three loci mismatches in six patients, and two loci mismatches in three patients. A HLA-nested polymerase chain reaction with sequence-specific primer typing demonstrated chimeric cells in eight of nine donors. The conditioning regimen consisted of CA/CY/TBI in six patients, BU/CY in one patient, and Flu/L-PAM in two patients. The median number of CD34+ cells infused was

$2.25 \times 10^6$ /kg. The prophylaxis against graft-versus-host disease (GVHD) was tacrolimus with minidose methotrexate (5 mg/kg, on days 1, 3, and 6). Engraftment was obtained in all patients. An acute GVHD of less than or equal to grade 2 developed in all patients except one who developed tacrolimus encephalopathy. Chronic GVHD developed in four of seven evaluable patients. Four patients died; one with fungal pneumonia, one with GVHD after cessation of tacrolimus because of relapse and two with disease progression. Five patients survived, with one patient being in complete remission (+75~+648).

These observations suggest that haploidentical SCT based on the fetomaternal immunological tolerance without TCD is possible.

#### (O1-4) MHC modulation of immune response across the leprosy spectrum.

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Following our demonstration on the involvement of Arg<sup>13,70-71</sup> residues in pocket 4 of HLA-DRB1 molecule in tuberculoid leprosy (BT/TT), we have now conducted a comprehensive high resolution analysis involving 247 unrelated North Indian leprosy subjects. A positive association of HLA-DR\*15 ( $P < 7.52 \times 10^{-11}$ ) was confirmed in both BT/TT and BL/LL forms of leprosy. However, while DRB1\*1501 ( $P < 3.08 \times 10^{-8}$ ) was significantly associated with the BL/LL leprosy, DRB1\*1502 was significantly increased in the BT/TT form ( $P < 4.11 \times 10^{-3}$ ). Analysis of the residues in the hypervariable peptide binding region revealed increased relative risk towards BL/LL leprosy conferred by V<sup>86</sup>, Arg<sup>13</sup> and Pro<sup>11</sup> spread over pockets 1, 4 and 6 respectively. Our studies suggest that the presence of V<sup>86</sup> in pocket 1, known to be crucial for peptide binding might be responsible for decreased immune responsiveness and bacterial clearance, leading to BL/LL forms of leprosy. To further explain the differential association, observed in two polar forms of leprosy, functional studies revealed a significant overrepresentation of MT<sub>1</sub> cells (CD45RA- CD62L- CD11a<sup>bright</sup>,  $\gamma$ -Ifn producers) among BT subjects and MT<sub>2</sub> cells (CD45RA- CD62L+ CD11<sup>dim</sup>, IL-4 producers) in BL/LL group. Also, 1501/02 +ve patients had significantly increased MT<sub>1</sub> representation than the negative group ( $p < 0.001$ ). This data indicates immune dynamics in leprosy and provides evidence that functional immune response is dictated by the presentation of relevant peptides of *M. leprae* by the host MHC.

#### (O1-5) Characterization of HCMV-specific CD8 T cells using HLA-A\*02 tetramers

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A significant number of cytomegalovirus (HCMV)-specific CD8+ T cells are found in PBMC from latently HCMV-infected healthy donors. We characterized HCMV-specific CD8+ T cells in the present study. We used HLA-A\*0201 and HLA-A\*0206 tetramers including HCMV pp65-495-503 epitope-peptide to identify HCMV-specific CD8+ T cells as well as mAbs specific for differentiation and maturation makers CD27, CD28 and CD45RA to distinguish both differentiation and maturation stages of HCMV-specific CD8+ T cells. Multi-color flowcytometric analysis of HCMV-specific CD8+ T cells showed that they predominately express CD27- or low CD28-CD45RA+ or -phenotypes. Whole CD8+ T cells expressing CD27-CD28-CD45RA+ or -showed high level of perforin expression while those with CD27<sup>low</sup>CD28-CD45RA+ or -showed moderate level of perforin, suggesting that CD8+ T cells expressing these phenotypes have cytolytic activity. Indeed, HCMV-specific CD8+ T cells expressing these phenotypes effectively killed target cells pulsed with HCMV pp65-495-503 epitope-peptide. CD8+ T cells expressing CD27-CD28-CD45RA+ showed lowest proliferation activity, highest susceptibility to anti-CD3 mAb-induced apoptosis and highest expression of perforin, strongly suggesting that CD8+ T cells expressing this phenotype are terminal effector T cells. On the other hand, CD8+ T cells with CD27<sup>low</sup>CD28-CD45RA+ or - and CD27-CD28-CD45RA- may have characteristics of memory/effector T cells since they have proliferation ability. These results indicate that in healthy donors HCMV-specific memory CD8+ T cells are always stimulated by HCMV and then mature to memory/effector or effector cells.

#### (O1-6) HLA-based human papillomavirus epitope prediction

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We previously reported HLA class II DRB1\*1602 allele to confer susceptibility to infection with HPV types that belong to the subgroup A9 (HPV-16, -31, and -58). As peptides that bind to a given major histocompatibility complex (MHC) molecule share sequence similarity, using a computer algorithm (RANKPEP) (available on-line at [www.mifoundation.org/Tools/rankpep.html](http://www.mifoundation.org/Tools/rankpep.html)) we examined E2, E6, E7, and L1 protein sequences of HPV-16, -31, and -58 for the prediction of peptide sequences which are likely to bind to the susceptibility HLA DR2 molecule. No common predicted peptide from the analysis of regulatory protein E2, or transforming proteins E6 and E7 sequences could be identified. However, two 9 aminoacid residue peptides within the major capsid protein L1 of HPV-16, -31, and -58 were predicted as being capable of binding to HLA DR2. The amino acid sequence of the two predicted peptides were AQQHNNGIC, and KFGFPDTSF.

MHC class II are present only on antigen-presenting cells, bind exogenously derived peptides, and are recognized by CD4 helper T-cells. Prediction of peptides that can bind to MHC molecules is important for selection of peptides capable of eliciting an effective immune response. The fact that common epitope candidates could be localized only within the L1 sequences, agrees with the concept that capsid antigenicity seems to play an important role in HPV infection and related diseases. As vaccines appear to be a promising way of preventing cervical cancer, computer based scanning for possible epitopes may be time and cost saving for future experiments aimed to evaluate successful HPV vaccines candidates.

### **(O2-1) Preliminary investigations of the HLA class I antigens expression on the epithelial cells of the nasopharyngeal carcinoma in Viet Nam**

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The non expression of HLA class I molecules on the tumoral cells is considered as a mean of the malignant process to evade the host immune response.

The expression of these HLA antigens was analysed in 20 undifferentiated nasopharyngeal carcinoma (UCNT) patients biopsies by immunofluorescent technic with antibodies kindly given by Dr. Brian Tait (Royal Tissue Typing Laboratory Melbourne Hospital Australia).

The loss in expression of the pan antigens (ABC) was found in 16 cases (80%) and the b2m in 15/20 (75%); of both in 14/20 (70%). At the locus specific antigens this frequency is lower: 65% for the locus A and 45% for the B. The allelic specific antigens were not present in 75% for the allele A and 60% for the B. When they were detected, the most frequently encountered were Bw4 (25%), B7 (20%) and A2 (15%) A28 (15%).

The percentage of non expression seemed higher than in other malignant tumors from the other sites mentioned in the literature, perhaps it may be due to the disease late stage of detection in the majority of the patients (T2. and T3) and perhaps also in the nasopharyngeal carcinoma the Epstein Barr virus infection is always one cofactor if not the cause frequently discovered (high titre of antibodies against early antigens of the virus

### **(O2-2) Microsatellite analysis of the HLA region in head and neck squamous cell carcinoma patients using DNA pools.**

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The HLA region on chromosome 6 contains up to 300 genes of which many are highly polymorphic. Microsatellites are useful markers in association-studies to identify disease-associated regions or genes. Previous research on head and neck squamous cell carcinoma (HNSCC) indicated the HLA-B40-DR13 haplotype to be significantly associated in Dutch HNSCC patients. We used 69 microsatellites to refine the association with the B-DR region and to localize genes associated with HNSCC. Four different DNA pools were made: 1 control DNA pool (N = 106) and three patient DNA pools according to the localisation of the tumor. An oral cavity DNA pool (N = 159), a larynx DNA pool (N = 101) and an oropharynx DNA pool (N = 86). These pools were tested for all 69 microsatellite markers in the HLA region. Thirty-nine microsatellites were located in the class I region, 22 microsatellites in the class II region and 8 microsatellites in the class III region. One universal PCR program was performed for these microsatellites and PCR products were analysed by Gene Scanning on an ABI 3700 Sequencer. Preliminary results show two regions with significantly associated markers: the HLA-DQ-DR region and the MICA-HLA-B-C region. Among the significant microsatellites, particularly observed in the oral cavity DNA pool, the MICA microsatellite association was studied in detail.

### **(O2-3) MHC Class I association with asthma in the Busselton population.**

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Asthma occurs more commonly in atopic (allergic) individuals and is characterised by variable and excessive airway narrowing, airway inflammation and alterations of airway dimensions - remodelling. A number of studies have shown associations between the MHC region and phenotypes including positive skin tests to common allergens and total serum IgE. However, studies of candidate genes within the MHC have been limited to genes around the Class II region and TNF gene cluster. It is not known whether any genes in these regions are directly involved in the pathogenesis of asthma or related phenotypes. To identify susceptibility loci for asthma and related phenotypes

within the MHC, associations with a set of well defined polymorphic microsatellite markers across part of the MHC region were studied in 82 cases with asthma and 94 healthy controls from the Busselton population in Western Australia. Statistically significant differences in allele distribution were found between cases and controls within two polymorphic microsatellite loci telemeric to HLA-A. ( $p = 0.023$  and  $p = 0.0033$  respectively). In addition, the allele frequencies for A5 of MICA was lower in cases of asthma (7/164–4.27%) compared with healthy controls (25/188–13.3%) ( $p = 0.003$ ). Further studies are underway to evaluate the potential association between candidate genes in the Class I region and asthma.

#### **(O2-4) Association of susceptible genes to Japanese cedar pollen allergy using SNPs in LHA gene region**

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Using methods developed to examine single nucleotide polymorphisms in the human MHC, we have undertaken the study of cedar pollinosis and possible genetic with the MHC. Blood samples were collected from 132 unrelated patients with Japanese cedar pollen allergy and from 177 persons without any symptoms of allergy for use as healthy controls. All samples were titrated for the presence of specific IgE antibodies to Japanese cedar, orchard grass, mugwort pollen, and mite by RAST. In addition, 165 SNPs from the HLA region were typed by SSP assays as well as determining each of the HLA-A, -B, -DR types of all samples. The results of these tests showed that all patients were positive for IgE antibody to Japanese cedar pollen and some were positive for the other allergen. Ninety-one healthy control samples were negative for all of the specific IgE antibodies tested, while 86 controls had one or more specific IgE antibody while they did not show any symptoms. The SNPs data were analyzed for the association with pollinosis using two sets of controls, those that were completely IgE negative and IgE positive controls. When the IgE negative controls were used, 10 SNPs near the HLA-A region showed a significant association with a chi-square value of approximately 6. However, when the IgE positive controls were used, no association was detected. This results indicate that about half of healthy Japanese may have some susceptibility to pollinosis and that there may exist susceptibility genes may exist near the HLA-A region.

#### **(O2-5) Heterozygote disadvantage of HLA-DRB1\*0405 and DRB1\*0901 in susceptibility of rheumatoid arthritis in the Korean population.**

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The present study was undertaken to elucidate the impact of specific DRB1 alleles in conferring disease susceptibility and severity for RA in Koreans. The patients with RA ( $n = 576$ ) and controls ( $n = 392$ ) with same ethnic background were included in this study. HLA-DRB1 typing were performed by polymerase chain reaction and direct DNA sequencing analysis. Statistical analysis of the HLA typing data revealed that the frequencies of DRB1\*0405 (24.4% vs. 7.1%) and DRB1\*0901 (14.4% vs. 8.2%) were significantly higher in patient group than in control. The patients and controls were then divided into 6 groups based on the possession of DRB1\*0405 and DRB1\*0901. Re-analysis of the data on these groups appeared that frequency of DRB1\*0405/DRB1\*0901 heterozygote [ $p = 0.0001$ , OR 58.5 (95% CI 7.99–427.7)] was much higher in patient group than in control. The odd ratio of the heterozygous group was even higher than in DRB1\*0405 homozygous group (58.5 vs. 37.2). The age at disease onset was significantly lower in patients carrying HLA-DRB1\*0405 than in those without HLA-DRB1\*0405 [ $p = 0.005$ ,  $36.3 \pm 11.9$  vs  $39.2 \pm 12.0$ , respectively]. In addition, the radiographic changes (stages 2–4) were more frequent in patients carrying HLA-DRB1\*0405 allele only [ $p = 0.012$ , 91.1% vs 83.7%, OR 2.0 (95% CI 1.17–3.41)]. These data suggest that both DRB1\*0405 and DRB1\*0901 are strongly associated RA susceptibility and DRB1\*0901 has additive disease susceptible effect. However, only the DRB1\*0405 is associated with disease progress and severity.

#### **(O3-1) An approach to minimize DRB1 high-resolution ambiguity in Japanese population using SSO probes that establish a linkage of two polymorphic sequences.**

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The reverse SSO method suffers lower resolving power compared to SSP and SBT methods. This is due to the fact that certain HLA genotypes contain identical polymorphism, but differ only in their arrangement, cannot be resolved without establishing a polymorphism linkage. Since a single SSO probe designed for a single target sequence cannot establish a linkage of multiple polymorphic sequences on

a strand of DNA, it has remained as inherent ambiguity problem in the SSO method.

Here, we demonstrate that selected DRB1 probes that establish linkage of two or three distant sequences are able to resolve ambiguities typically seen in Japanese population. The ambiguous combinations in DRB1 locus studied include well-represented alleles in Japanese population such as: [DRB1\*04031/04/06, DRB1\*13021] = [DRB1\*04071, DRB1\*13011]; [DRB1\*0405, DRB1\*1403] = [DRB1\*04051, DRB1\*1412]; [DRB1\*130201, DRB1\*140101] = [DRB1\*130101, DRB1\*140701] etc. Overall effects of newly developed DRB1 probes in routine typing results in Japan will be discussed.

Representative ambiguities that can be resolved by this technology are: A\*2501, 02 specific probe to distinguish A\*03/A\*25 and A\*32/A\*66, A\*25/A\*74 and A32\*/A\*66; probes specific for two polymorphisms to resolve Bw4 associated ambiguities, B4901/B4002 = B4019/B50011, B4901/B7805 = B520101/50011 ambiguities in B-locus; DRB1\*0308/1107 specific probe to exclude DRB1\*0308 from common DRB1\*11 and DRB1\*03 alleles; CW\*0106 specific probe to exclude all CW\*0106 ambiguities.

### **(O3-2) Typing for the C282Y and H63D mutations of HFE by pyrosequencing**

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Pyrosequencing is a rapid technique suitable for sequence analysis of short regions of PCR amplified DNA, and particularly suited for SNP analysis. It makes use of pyrophosphate generation upon incorporation of nucleotides during primer extension as dNTPs are added in a pre-determined order. The generation of pyrophosphate is coupled to a luciferase catalysed reaction resulting in light emission if the particular dNTP added is incorporated, yielding a distinctive pyrogram. In this study we compared Pyrosequencing to FRET/Melting curve analysis on the Roche Lightcycler for HFE C282Y and H63D genotyping.

The Pyrosequencing protocol comprised of capture of PCR amplicons on streptavidin-coated beads, isolation of single-stranded DNA, hybridization to a sequencing primer and analysis by Pyrosequencing.

Results by both methods were completely concordant, and included 41 CC, 19 CY, 16 YY, 38 HH, 25 HD, and 13 DD samples. None of the results were ambiguous and all genotypes were automatically and correctly assigned by the manufacturer & acute;s software. In routine practice, the method has proved to be reliable, and because genotype assignment is based on pattern recognition, the approach is insensitive to differences in product yield for different samples. The instrument can analyse 96 samples/10 minutes and has the flexibility to analyse a variety of different SNPs on the same analytical run. Assay design and set-up for HFE genotyping, as for numerous other SNPs also developed, was a very simple proposition. These qualities make Pyrosequencing highly suitable for SNP analyses for medium to large size laboratories, and for both diagnostic and research applications.

### **(O3-3) KIR phenotype diversity generated by allelic diversification within limited genotype distribution in Japanese**

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Stanford University

Killer cell Immunoglobulin-like Receptors (KIR) are a highly diverse family of cell surface receptors which regulate NK cells and subsets of T cells through recognition of HLA class I molecules. The number and combination of *KIR* loci (genotypes) has been found to differ greatly amongst human individuals and also between ethnic populations.

A unique distribution of only ten *KIR* genotypes was observed in >100 healthy unrelated Japanese individuals, originating from Hokkaido to Okinawa. The four most frequent genotypes accounted for 94% of the panel, due mainly to the high frequency [80%] of one specific haplotype group, the group A haplotypes. No other population group studied displays such a restricted pattern in *KIR* genotype at the gene content level.

Despite the limited genotype diversity, expression patterns of *KIR* in the Japanese panel as assessed by flow cytometry with six anti-KIR antibodies, were highly diverse and comparable to a Caucasoid panel, indicating that the limited variability of *KIR* gene content does not equate with a constricted phenotype of *KIR* repertoire. High resolution *KIR* typing by full-length sequencing of cDNA transcripts revealed allelic distribution to correlate with these phenotypes, suggesting allelic diversification as the mode generating *KIR* diversity in this population, and occurring mainly in the group A haplotypes. Also supporting this view was the discovery in this panel of several novel *KIR* alleles with potential for unique ligand specificity.

### (O3-4) Polymorphic alu insertions within the MHC class I genomic region

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Many polymorphic Alu insertions (POALINs) belong to a subgroup of the Alu multicopy retrotransposon family of short interspersed nucleotide elements (SINEs) that are categorized as AluYb8. The number of AluYb8 members (~500–2000 copies) is significantly less than the fixed ~0.5 million Alu copies per haploid genome in humans. Because of their activity and mobility, POALINs are estimated to contribute to at least 0.4%–1.0% of human genetic disorders.

We study the Major Histocompatibility Complex (MHC) class I region on the short arm of chromosome 6 because this region has a high gene density, many genes with immune system functions, large sequence variations and diversity, duplications and redundancy, and a strong association with more than 100 different diseases. Since very little is known or understood about POALINs within the MHC genomic region, we undertook to identify some of the members of the AluYb8 subfamily and to study their frequency distribution and genetic characteristics in different populations. As a result of our comparative genomic analyses, we have successfully identified the insertion sites for 5 POALINs distributed within the MHC class I region. This presentation will outline the locations of the insertions and sequence features of the 5 MHC POALINs, their single site and haplotype frequencies in a few examples of different geographic populations, and their association with different HLA class I genes and disease. Our findings suggest that the MHC POALINs have a potential value as lineage and linkage markers for the study of human population genetics, disease associations, genomic diversity and evolution.

### (O3-5) The origin of MHC involvement in allograft rejection

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Fish are the most primitive species with MHC class I and II genes. Whereas in the elasmobranchs (shark and ray) the class I and II loci are linked, in teleost fishes they are not. We showed that the MHC in shark is an important determinant for the vigor of allogeneic skin graft rejection. Cardwell and coworkers showed that the class II linkage group is an important determinant for allograft rejection in the teleost Gila topminnow, and we recently showed the same for the class Ia linkage group in rainbow trout. Probably allograft rejection in fish has more functional importance than in mammals: Fish skin epidermis consists of living cells, many fish species are cannibalistic, and cell grafting in water should be easier than in air. However, whereas in mammals it is thought that MHC mediated allograft rejection and pathogen recognition depend on similar mechanisms, namely, small changes in the MHC or MHC/peptide structure, this may not be true in fish. The relative importance of ‘Danger’ signals for immune stimulation is probably more important than in mammals, because fluctuating body temperatures alone already should influence MHC-TCR binding efficiency. Interesting in this respect is that rainbow trout have an unusual high level of allelic class Ia variation extending far beyond the binding groove.

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### (O3-6) Comparative analysis of MHC regions elucidated by genomic sequencing

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We previously reported the genomic sequence of the 2.2 Mb HLA class I and III regions, and also of one of the HLA paralogous regions, the 1.1 Mb CD1 gene region on chromosome 1. As a next step to comparative genome analysis, we are now investigating gene organization of the MHC or ancestral MHC regions by genome sequencing in various species in order to elucidate molecular mechanism for the evolutionary process of the MHC regions. To attain this goal, we have so far determined the genomic sequences of 11 Mb in total of the MHC or ancestral MHC regions from following species: 511 kb from amphioxus, 271 kb from shark, 209 kb from rainbow trout, 243 kb from quail, 3.8 Mb from rat, 1.1 Mb from pig, 3.3 Mb from rhesus macaque, 1.75 Mb from chimpanzee, and compared to each other among species. In summary, these analyses, the basic structure of the MHC region is largely conserved among various species, but always remodeled as responses to environmental pathogens by “birth and death” of MHC and MHC-linked genes. Further the comparative genome analysis of the MHC regions provides us with informative data which help understanding the evolutionary process of the human genome.

### (O3-7) Comparative genomic analysis between a chimpanzee sequence and two human sequences with different HLA haplotypes within the entire MHC Class I region.

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The chimpanzee shares nearly 99% sequence identity with the human genome. Our aim is to determine the genomic sequence in the chimpanzee MHC class I region and to compare the genomic diversity between the two species. This report presents the first large scale comparison between the human and the chimpanzee genomes via the sequence analysis. This 1.75 Mb stretch of DNA which encompasses the entire class I along with the telomeric part of the MHC class III regions corresponds to an orthologous 1.87 Mb of the human HLA region. Sequence analysis confirms the existence of a high degree of sequence similarity between the two species. However and importantly, this 98.6% sequence identity drops to only 86.7% once taking into account the multiple insertions/deletions (indels) dispersed throughout the region. And, when divided into MHC and non-MHC genes, the nucleotide identity was 99.3% for 28 non-MHC genes and 97.1% for 6 MHC genes and 1 MIC gene. This difference between non-MHC and MHC genes is probably due to balancing selection or over-dominant selection operating through recombination to maintain polymorphism in functional MHC genes. In this comparative analysis, diversity profiling, indel distribution, pattern of nucleotide substitution, tendency toward diversity between MHC (multi-copy) and non-MHC (single-copy) genomic regions will be presented using genomic sequences the entire MHC class I region from 2 different HLA haplotypes and 1 chimpanzee sequence.

### (O3-8) Sorting out the orthology among hominoid MHC-A related loci: genomic analysis of class I genes of the orangutan (*Pongo pygmaeus*)

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Stanford University

Study of hominoid species provides evidence for several genes related to *HLA-A*. Whereas humans have *HLA-A* and *HLA-H*, chimpanzees have *Patr-A*, *Patr-H*, and another *A*-related gene, *Patr-AL*. Phylogenetics show *HLA-A*, *Patr-A* and *Gogo-A*, present in human, chimpanzee and gorilla, are orthologues. Orthology seems to dissolve with orangutan *MHC class I* genes, for which the sequence designated as *Popy-A*, does not cluster with *HLA-A* but with *Patr-AL*.

We aimed to determine whether orangutans have an *HLA-A* orthologue. *MHC class I* genes isolated from an orangutan cosmid library were characterized. A clone corresponding to the *HLA-A* containing region was shown to be conserved with regard to gene order, orientation and intergenic sequences. This genomic analysis identified a new *Popy class I MHC* gene, that is orthologous to *HLA-A* and distinct from either the previously assigned *Popy-A* gene or the polymorphic pseudogene, *Popy-Ap*. The new gene is closest in sequence to the gibbon class I gene *Hyla-A*. Preliminary study suggests the orangutan ortholog of *HLA-A* has functional polymorphism.

In conclusion, phylogenetics shows the polymorphic gene named *Popy-A* is orthologous to the non-polymorphic *Patr-AL*, a gene not present in humans. Secondly, the polymorphic *Popy-Ap* pseudogene, appears orthologous to human and chimpanzee pseudogenes *HLA-H* and *Patr-H*. Most importantly, a newly discovered orangutan class I locus is orthologous to *HLA-A*. Thus, at least three genes in the *MHC-A* family were present in the ancestor of modern hominoids. This study demonstrates how class I genes can change their character, function and polymorphism in the course of evolution.

## Poster

### (P1-1) Rainbow trout MHC class Ia and Ib regions; Interlocus recombination created extreme high levels of Ia diversity

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Although rainbow trout only has a single class Ia locus, the diversity of Ia sequences is unprecedented. Homology levels between allelic trout a1 or a2 domains can be as low as when compared with mammalian sequences. Copies of most of the very diverse trout Ia a1 and a2 lineages can be found elsewhere in the genome. We identified a class Ia and a class Ib region on two different trout chromosomes, possibly originating from the previous tetraploid state of salmonids. This arrangement seems to allow genetic exchange between the Ia locus and a variety of class Ib loci, resulting in the enormous Ia allelic diversity.

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### (P1-2) The diversity of bovine MHC class II DRB3 genes in Japanese Black, Japanese Shorthorn, Jersey and Holstein cattle in Japan

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We sequenced exon 2 of the MHC class II *DRB3* gene from 471 individuals in four different Japanese populations of cattle (201 Japanese Black, 101 Holstein, 100 Japanese Shorthorn, and 69 Jersey) using a new method for sequence-based typing (SBT). We identified the 34 previously reported alleles and four novel alleles. These alleles were 77.9% to 100.0% identical at the amino acid level to the bovine *MHC (BoLA)-DRB3* cDNA clone NR1. Among the 38 alleles, eight alleles were found in only one breed in this study. However, these alleles did not form specific clusters on a phylogenetic tree of 236bp nucleotide sequences. Furthermore, these breeds exhibited similar variations with respect to average frequencies of nucleotides and amino acids, as well as synonymous and non-synonymous substitutions, in all pairwise comparisons of the alleles found in this study. By contrast, analysis of the frequencies of the various *BoLA-DRB3* alleles in each breed indicated that *DRB3\*1101* was the most frequent allele in Holstein cattle (16.8%), *DRB3\*4501* was the most frequent allele in Jersey cattle (18.1%), *DRB3\*1201* was the most frequent allele in Japanese Shorthorn cattle (16.0%) and *DRB3\*1001* was the most frequent allele in Japanese Black cattle (17.4%), indicating that the frequencies of alleles were differed in each breed. In addition, a population tree based on the frequency of *BoLA-DRB3* alleles in each breed suggested that Holstein and Japanese Black cattle were the most closely related, and that Jersey cattle were more different from both these breeds than Japanese Shorthorns.

### (P1-3) Comparing of antigen binding groove of MHC class II DR molecule of cattle and human

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The major histocompatibility complex (MHC) polymorphism occurs predominantly at residues involved in peptide binding, and there is compelling evidence that the polymorphism is maintained by some form of balancing selection. The essential role of the MHC molecules for immunological recognition of foreign peptide antigens implies that the cause for this selection is related to the influence of MHC polymorphism on host defense against pathogens. To study the function of bovine MHC (*BoLA*)-DR molecules, we detected positive and negative selection of *BoLA-DRβ1* and human MHC (*HLA*)-DR & *β1* domain and compared these. First, we compared the average of amino acid, nucleotide, nonsynonymous and synonymous substitutions in pairwise of 103 *BoLA-DRB3* and 236 *HLA-DRB1* alleles. The result shows that the rates of nonsynonymous substitution in *BoLA* were higher than that of *HLA*. Next, we detect positive and negative selection at single amino acid sites in *BoLA-DRβ* or *HLA-DRβ* domain by a new method of Suzuki and Gojobori. In *HLA*, 9 positive and 6 negative selection sites were detected and in *BoLA*, 7 positive and 2 negative selection sites were detected. In both of human and cattle, positive selection sites were mainly existed in antigen recognition site (ARS) site. Whereas, three negative selected sites were detected in

ARS of HLA, but not in ARS of BoLA. These result shows that the function of single amino acid were very close, but some sites have the different function in human and cattle.

#### **(P1-4) Sequences and diversity of 17 new ovine MHC class II DRB1 alleles from three breeds of sheep**

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To investigate the genetic diversity of the sheep MHC (Ovar) class II *DRB1* locus, we amplified exon 2 of *Ovar-DRB1* alleles by the polymerase chain reaction (PCR) and determined the nucleotide sequences of both resultant strands after cloning. In our study of a total 97 sheep of three individual breeds, namely, Suffolk, Cheviot and Corriedale, we identified 18 previously published alleles and 17 new alleles. These alleles were 83.4% to 94.1% identical at the nucleotide level and 71.4% to 90.9% identical at the amino acid level to *Ovar-DRB1\*0101*. We identified six new alleles in Cheviot sheep and eleven new alleles in Suffolk sheep. Furthermore, we identified 15, 6 and 1 alleles in Suffolk, Cheviot and Corriedale sheep, respectively, that have only been found in these breeds to date. Analysis of the frequencies of the various *Ovar-DRB1* alleles in each breed indicated that *Ovar-DRB1\*0702* was the most frequent allele in Suffolk sheep (23.9%), *Ovar-DRB1\*0203* was the most frequent allele in Cheviot sheep (27.5%) and *Ovar-DRB1\*0201* was the most frequent allele in Corriedale sheep (25.0%). A comparative analysis of the positions of polymorphic residues in the first extracellular domain of the DRB genes of sheep, man and mouse revealed the extraordinary similarity among positions of polymorphic residues that are associated with the antigen-recognition site (ARS). Moreover, the extent of polymorphism seems to be similar in sheep, human and mouse.

#### **(P1-5) Polymorphism of MHC Class II Alleles associated with Immune Response in Bovine Leukemia Virus Infection**

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In a previous study, we identified that the alleles of the ovine leukocyte antigen (*OLA*)-*DRB1* gene that encode the Arg-Lys (RK) motif and the Ser-Arg (SR) motif at positions  $\beta^{70/71}$  of the *OLA-DRB1* domain are associated with resistance and susceptibility, respectively, to development of bovine leukemia virus (BLV)-induced ovine lymphoma. Here, we investigated the different immune response in sheep that had the RK/RK or SR/SR from early stages of onset of the disease until 30 weeks after infection with BLV. Although the viral load had been maintaining low levels throughout the experimental period, the sheep with the RK/RK genotype could induce expansion of CD5-B-cells and rapid production of neutralizing antibody in the early phase of infection. The level of incorporation of [<sup>3</sup>H]thymidine by peripheral blood mononuclear cells from the sheep with RK/RK genotype gave a strong response to BLV virion antigen and synthetic antigenic peptides that corresponded to T-helper epitope of the BLV envelope glycoprotein gp51. In contrast, the sheep with SR/SR genotype showed a strong response to BLV virion antigen and synthetic antigenic peptides that corresponded to T-cytotoxic and B-cell epitopes. In such cases, the animals with the RK/RK strongly expressed IFN- $\gamma$ , the animals with SR/SR genotype strongly expressed IL-2. Moreover, we found that these proliferating cells were MHC-restricted CD4<sup>+</sup>T-cells.

#### **(P1-6) Analysis of genomic structure in the swine leukocyte antigen (SLA) class I region by large scale genome sequencing.**

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Genome analysis on the swine leukocyte antigen (SLA) region will give important information from a viewpoint of a possible strong candidate of pig organs as a donor for xenotransplantation. Here, we carried out large scale genome sequencing of the SLA class I region and compared the genomic structure between the pig and human MHCs in order to investigate gene structure of the SLA region, such as accurate number of expressed genes, gene order and genomic diversity.

A contig map of the SLA class I region spanning about 1.1 Mb was constructed by YAC and BAC overlapping clones. Genomic sequences of ten BAC clones carrying the entire SLA class I region were determined by the shotgun method. These clones included a total of 1,162 kb genome segment between the *LTB* gene in the class III region (most centromere-side) and the *UBD* gene (most telomere-side) in the class I region. The genomic sequences thus obtained in swine were compared to those of the corresponding human MHC segments. As a result, the length of the SLA class I region is about 890 kb shorter than the HLA class I region. Contrasting the HLA class I region, 158 kb

and 307 kb segments including the non-classical and classical SLA class I gene clusters, respectively reside separated by a 611kb segment. However, the nucleotide sequence comparison between the human and swine reveal that the gene organization of non-MHC genes except the MHC class I genes is remarkably similar between the two species.

### **(P1-7) Genetic polymorphism of the swine major histocompatibility complex (SLA) class I genes, SLA-1, -2 and -3**

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The swine major histocompatibility complex (*SLA*) encodes polymorphic molecules involved in the genetic control of immune response and allo- and xeno-graft rejection. In order to compare with the genetic polymorphisms and allelic variations of the *SLA* class I genes to those of the *HLA* class I genes, RT-PCR products of the second and third exons of the three *SLA* classical class I genes, *SLA-1*, -2 and -3 were subjected to nucleotide determination. These analyses allowed the identification of 19 alleles including 13 novel alleles in 32 individuals from three different breeds of miniature swine, Göttingen, Clawn and Mexican hairless pigs, and one mixed breed. Construction of a phylogenetic tree using the nucleotide sequences of those 19 alleles indicated that the *SLA-1* and -2 genes are more closely related to each other than to *SLA-3*. Selective forces operating at single amino acid sites of the *SLA* class I molecules were analyzed by the Adapsite Package program. No positive selection site was found at the putative non-antigen recognition sites (ARSs). However, among the 11 positively selected sites observed in the *HLA* classical class I molecules, only three corresponding positions in the *SLA* class I molecules were inferred as positively selected. Further, as many as four amino acids at the putative ARSs were identified as negatively selected in the *SLA* class I molecules, suggesting that several functional sites for antigen and cytotoxic T lymphocyte (CTL) recognition by the *SLA* class I molecules may be different from those of the *HLA* class I molecules.

### **(P1-8) Information systems in tissue typing: a low cost, customised laboratory management system in use in Sydney, Australia**

Zlatibor Velickovic

Australian Red Cross Blood Service

In the present day and age, information systems play a crucial role in laboratory management. Specific needs of tissue typing laboratories are often not envisaged by the large laboratory information systems. With the requirements of clients and staff increasing with time, a low cost laboratory management system was developed for the Sequencing Laboratory in Sydney, to automate and document increasingly complex laboratory procedures. The system consists of several custom made modules utilising low cost hardware and software resulting in a versatile, multifunctional and cross platform solution. The system electronically automates and archives the following procedures: specimen list acquisition, automated test assignment based on HLA type, automated PCR worklist generation, electronic gel documentation, automated sequence worklist generation based on PCR outcome, archiving of worklist, automated statistics online and results status monitor online. These modules and procedures are discussed in more detail in the presentation. In conclusion the system in use in Tissue Typing, Sydney, Australia is a low cost hardware and software solution for complex lab management tasks. It is relatively easy to produce in a customized format making it applicable to a variety of laboratory procedures.

### **(P1-9) Whole genome amplified samples can be typed for single nucleotide polymorphisms using fluorescence correlation spectroscopy measurement**

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Whole genome amplification (WGA) methods were adopted for SNP typing to save the amount of genomic DNAs that have to be typed for thousands of different SNPs. By the WGA, the entire genome is randomly amplified using a random or a degenerated primer. Five to 10ng of the genomic DNAs were amplified by a WGA method (I-PEP-PCR or DOP-PCR). Using 1/100 to 1/500 amounts of the WGA products as templates, subsequent PCR analyses were successfully performed. SNPs were genotyped by PCR-sequence specific primer (PCR-SSP) method followed by fluorescence correlation spectroscopy (FCS) measurement (Olympus Optical). Since movements of DNA fragments in a solution depend on their sizes, primers (smaller molecules) move faster than PCR-SSP-amplified fragments (larger molecules) in the PCR product solution. After a narrow laser beam spots DNA fragments at a very small area (1 femtoliter) in the solution, the signals of fluorescence-labeled molecules are detected by a highly sensitive spectrometer, then the numbers and sizes of both primers and amplified

fragments are determined. The typing results were evaluated for four different SNPs on tumor necrosis factor- $\alpha$  receptor 1 or 2 gene (TNFR1 or TNFR2). Genotypes determined by the PCR-SSP-FCS method using WGA products were in 100% concordance with those typed by sequencing using genomic DNAs. We are currently performing 5,000 typings /day in average using WGA samples from patients with several common diseases. WGA methods coupled with the FCS system allow specific and high-throughput genotyping of thousands of small amounts of samples for hundreds of different SNPs.

### **(P1-10) A Pyrosequencing™ method for typing of HLA-A gene**

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[Aimed and Background] The HLA matching correlates with success rate of marrow and organ transplantation. The HLA typing is more and more significant recently. The SBT (sequencing based typing) has many advantages among HLA genotyping methods which have been developed for high resolution typing. However, the SBT has complicated processes. In addition, there are many ambiguous heterozygous genotype combinations in SBT. Pyrosequencing™ is a novel DNA sequencing technology which detects the DNA polymerase-catalyzed extension in real-time by detection of pyrophosphate release. This system has advantages that it is able to eliminate the ambiguity because of its ability of haplotyping, it does not need gel electrophoresis and DNA fluorescent labeling, and 96 samples are analyzed simultaneously on 96 well microplate. In this study, we have established a high resolution HLA typing system specialized for Japanese population using Pyrosequencing™.

[Methods] DNA samples of which HLA type were already identified as having allele with high frequency in Japanese were extract from B cell lines and blood samples of Japanese. The DNA fragments including SNP (single nucleotide polymorphic) sites in exon2 and exon3 of HLA-A gene were amplified respectively by PCR, and typed 25 SNPs using Pyrosequencing™ with 8 sequencing primers.

[Results] The SNPs types were obtained predictably in the most samples. It was also possible to type heterozygous samples without any ambiguity because of each separated peak. On these results, Pyrosequencing™ method will contribute to improve throughput and accuracy in Japanese specific HLA typing.

### **(P1-11) Diversity of HLA Class II in New Zealand Maori: the DR, DP and DQ alleles**

Mary Clare Tracey, John M. Carter

Wellington School of Medicine

New Zealand Maori are of Polynesian ethnicity, and like many Polynesian people have not been well-studied in terms of HLA allelic variation. We have used sequence-based typing to examine the Class II DR, DP and DQ loci of 50 individuals. We have detailed ancestry information for each individual that allows us to determine the most probable genetic degree of Maori ancestry. This information also allows us to consider the possible ethnic origins of particular alleles not common to Polynesian people. We will present an analysis of all alleles detected in each of the three loci, as well as a comparison to recently published data on four Pacific Island populations. In addition, we will examine theories of Polynesian migration and the recent availability of information from the Taiwanese Donor Registry in order to determine if our data fit within those paradigms. As our primary interest is in donor matching for bone marrow transplantation we will also briefly discuss the New Zealand Bone Marrow Donor Registry, current donor numbers and the percentage chance of a Maori person finding a fully-matched (6/6) or partially-matched (5/6) donor.

### **(P1-12) Leukaemia incidence in various ethnic groups in New Zealand and criteria for bone marrow transplant.**

Mary Clare Tracey, John M. Carter

Wellington School of Medicine

Allogeneic stem cell transplantation is a very expensive treatment and doctors need to make decisions on the best allocation of resources to ensure optimal outcomes. Decisions are made based on a number of criteria, including the age of the patient, the stage of the disease and the possibility of finding a suitable donor.

No study has been done in New Zealand (NZ) to ascertain the level of need for allogeneic stem cell transplant amongst the NZ Maori or other ethnic groups. However Velickovic and Carter (1999) showed that for NZ Maori and NZ Pacific Islanders there was a significantly reduced chance of finding a donor, compared to NZ Caucasians.

To identify ethnicity variables in the incidence and mortality rates of leukaemias in the NZ population, we analyzed 7 years of data from the NZ Cancer Registry, comprising all individuals diagnosed with a transplantable leukaemic condition. We examined age at onset, ethnicity, incidence rates and gender over the period. Our analyses answered the following questions: 1) Do NZ Maori, NZ Pacific Islanders

and NZ Asians have the same incidence of leukaemia as NZ Caucasians? 2) Do NZ Maori, NZ Pacific Islanders and NZ Asians have the same mortality rates from leukaemia as NZ Caucasians? 3) Are there differences in age at onset between the ethnic groups?

### **(P1-13) Distribution of HLA-A and B antigens in the national stem cell donor registry program**

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Stem cell transplantation has become the therapy of choice for many hematologic and immunologic disorders. At present, only 25% of patients have suitable HLA identical siblings. Patients who do not have an HLA-matched related donor can sometimes obtain an unrelated donor by searching volunteer registries.

Objective: In 2002, the Thai national stem cell donor registry program was established to facilitate donor search for patients lacking HLA identical siblings. This study illustrates the HLA-A and B antigen frequencies in the Thai population.

Materials and Methods: By the end of April 2003, 1,740 HLA-A and B typed samples from unrelated stem cell volunteer donors at the National Blood Centre, Thai Red Cross Society had been cumulatively registered. Twenty milliliters of venous blood from each donor was collected and typed using the standard microlymphocytotoxicity test and commercial typing trays. The typing results were calculated into gene frequencies.

Results: In this population, 26 HLA-A and 43 HLA-B antigens were found. Similar to other findings in the Thai population, the most common gene frequencies were HLA-A2 (27.59%), A11 (26.63%), A24 (16.97%), B46 (12.31%), B60 (10.75%) and B13 (8.05%). In addition, the less common gene frequencies were HLA-A23 (0.09%), A25 (0.03%), A66 (0.03%), A80 (0.03%), B41 (0.03%), B45 (0.07%), B49 (0.03%), B67 (0.20%) and B70 (0.06%).

Conclusion: We can conclude that increasing the national donor pool will benefit treatment by facilitating more efficient results in finding suitable HLA-matched donors, especially when the patients possess rare HLA antigens.

### **(P1-14) Identification of an unusual HLA-B\*27 variant in a potential solid organ transplant donor**

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New Zealand Blood Service

The deficit in the donor to recipient population has led to a move towards living donor renal transplants. In New Zealand, potential donors (both related and unrelated), are typed serologically for HLA Class I and typed by DNA methods for HLA Class II. Infrequently, samples may be HLA Class I DNA typed in order to resolve ambiguities or to define homozygotes.

The donor in this study was HLA-A and B typed serologically using in-house typing plates. Since the results indicated an HLA-B40 homozygote a sample was referred for DNA typing. Initial HLA typing results by PCR-SSOP (InnoLiPa) defined the B locus as HLA-B\*2712, \*4002/29/35/37/40/41 indicating presence of an allele that was not identified by serological typing. The sample was further tested by PCR-SSP (Dynal) and the B locus confirmed as HLA-B\*2712, \*4004/29/35/40.

HLA-B\*2712 has previously been described in the Caucasoid and Hispanic populations and was assigned by WHO in 1997. It has been shown to lack reactivity with B27 monoclonal antibodies and alloantisera but reacts with some B40/B60 monoclonal antibodies and alloantisera. It is associated with the Bw6 public epitope.

This finding has led to a more in depth scrutiny of samples defined as homozygotes serologically. It also has a clinical significance in transplantation as it is unclear whether B27 should be considered an avoid antigen in future transplants.

### **(P1-15) Flow cytometric detection of anti-HLA antibodies in renal transplantation**

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INTRODUCTION: Even a small amount of anti-HLA antibodies can cause graft failure due to antibody-mediated rejection in renal transplantation. Flow cytometric procedure has been developed as a highly sensitive method for detection. The purpose of this study was to compare flow cytometric method with complement-dependent cytotoxicity (CDC) test, and to assess its clinical value in renal transplant recipients.

METHODS: CDC-PRA and Flow-PRA were compared in 154 candidates waiting for cadaveric renal transplantation. All candidates were divided into two: possibly sensitized patients with a history of pregnancy (PR), blood transfusion (BT) or transplantation (TX) (Group A,

n = 109) and non-sensitized patients (Group B, n = 45).

RESULTS: 10.1% (14/139) of negative CDC-PRA(T) patients were positive for Flow PRA (Class I). 73.5% (25/34) of positive CDC-PRA(WB) were negative for Flow PRA (Class II). Flow PRA was useful to reduce false-negative rate in CDC-PRA(T) and false-positive rate in CDC-PRA(WB). Flow PRA-positive rates in candidates with a history of PG, BT and TX were 35%, 34% and 64%, respectively. Previous BT and TX were related to production of anti-HLA Class I and Class II/I antibody, respectively. No positive Flow PRA was observed in Group B.

CONCLUSIONS: Flow PRA should be applied not to all candidates on waiting list because of a larger cost, but to selected candidates with immunologically high risk factors. When Flow PRA is negative, direct crossmatch can be omitted immediately before cadaveric transplantation. However, if Flow PRA is positive, direct crossmatch using flow cytometry would be strongly recommended.

### **(P1-16) Mismatching for HLA-C encoded motifs that interact with a distinct killer inhibitory receptor and acute graft versus host disease.**

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Recent interest in HLA-C and transplantation has shown that HLA-C plays a role in modulating natural killer (NK) cell activity. The interaction of self-MHC with receptors on a subset of NK cells is crucial for the protection from NK cell-mediated lysis of target cells. In man, this interaction has been mapped to a dimorphic epitope at residues 77 and 80 on the alpha 1 domain of HLA-C, each epitope interacting with a distinct killer inhibitory receptor (KIR). Alloreactive NK cells can be generated against stimulators expressing the opposite motif to that of the responder. It is thought that this may have implications for unrelated and extended family haematopoietic cell transplantation, as recent reports have suggested that HLA-C mismatches may be detrimental to transplant outcome. To determine whether natural killer cells have a role to play in GVHD in stem cell transplantation, mismatching for the HLA-C encoded motifs that interact with KIRs, thus influencing NK cell allorecognition, was assessed. Molecular based typing was undertaken to determine the level of HLA-C matching in matched unrelated patient/donor pairs as well as extended family donors. Each individual was identified as being positive for the Asn77 and Lys80 (group 1) and/or Ser77 and Asn80 (group2) HLA-C molecules. It was then determined whether the HLA-C mismatched patient/donor pairs were also mismatched for this motif and whether this affected transplant outcome

### **(P1-17) Correlation between disparity for the novel minor histocompatibility antigen, ACC-1, and clinical outcome after hematopoietic stem cell transplantation from an HLA-identical sibling.**

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The novel minor histocompatibility antigen, ACC-1, is restricted by HLA-A\*2402 and formed by a single nucleotide polymorphism (G/A) on BCL2A1 gene, which is localized on 15q24.3–25.1 region. We established a simple ACC-1 DNA typing method and investigated the allele frequency in Japanese subjects using DNA from 96 unrelated individuals. We found that the allele frequency of Tyr at codon 19, (ACC-1 positive allele), was 0.516 and that of Cys was 0.489. The frequencies of recipient ACC-1 disparity were estimated as follows; 18% in the unrelated donors, 12% in the parent/offspring donors, 11% in the sibling donors. We next evaluated the association between the compatibility of ACC-1 and GVL/GVH effect after hematopoietic stem cell transplantation (SCT) from an HLA identical sibling. We compared the risk for developing severe acute GVHD ( $\geq$  grade III) between ACC-1-incompatible (IC) and compatible (C) groups among HLA-A\*2402 (+) recipients (N = 42) who had undergone SCT for hematological malignancies with cyclosporine-based GVHD prophylaxis. One (16.7%) of 6 patients in the group IC and 3 (8.3%) of 36 patients in the group C experienced grades III or IV acute GVHD [Odds ratio for IC vs C: 2.2 (95%CI 0.19–25.5), P = 0.91], suggesting that the incompatibility of ACC-1 was not strongly associated with development of severe acute GVHD. Although the number of patients is still small, currently no relapse has been observed in the group IC. Given hematopoietic cell-specific expression of ACC-1, these preliminary observations strongly suggest that ACC-1 could be a potent GVL target which facilitates an effective adoptive immunotherapy for patients with hematological malignancies without exacerbating GVHD.

**(P1-18) HLA-DRB3 allele frequencies and DRB1-DRB3 Haplotypic associations in Koreans**

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HLA-DRB3 alleles are present in close linkage disequilibrium with particular DRB1 alleles and DRB1-DRB3 haplotypic associations vary among different ethnic groups. We have investigated the frequencies of HLA-DRB3 alleles and DRB1-DRB3 haplotypic associations in 800 Koreans, registered as marrow donors to the Korea Marrow Donor Program. DRB3 genotyping was done on 447 samples carrying DRB3-associated DRB1 alleles (DRB1\*03, \*11, \*12, \*13, and \*14) using PCR-single strand conformation polymorphism (SSCP) method. DRB1 genotyping was done using PCR-SSO and PCR-SSCP methods. The allele frequencies of DRB3\*0101, DRB3\*0202 and DRB3\*0301 were 7.3%, 13.6%, and 12.0%, respectively, and we found one case of probable new allele (DRB3\*01new, 0.1%). DRB1-DRB3 haplotype frequencies (HF) for DRB1 alleles with frequency >0.5% were: DRB1\*0301-DRB3\*0202 (HF 2.3%), DRB1\*1101-DRB3\*0202 (3.2%), DRB1\*1201-DRB3\*0101 (4.2%), DRB1\*1202-DRB3\*0301 (2.7%), DRB1\*1301-DRB3\*0101 (2.2%), DRB1\*1302-DRB3\*0301 (9.5%), DRB1\*1401-DRB3\*0202 (3.1%), DRB1\*1403-DRB3\*0101 (0.8%), DRB1\*1405-DRB3\*0202 (3.2%), and DRB1\*1406-DRB3\*0202 (0.8%). Strong associations were observed between DRB3\*0101 and DRB1\*1201, \*1301, and \*1403; between DRB3\*0301 with DRB1\*1202 and \*1302; between DRB3\*0202 and remaining DRB1 alleles. Most of the DRB1 alleles were exclusively associated with particular DRB3 alleles with relative linkage disequilibrium (RLD) values of 1.00, except for DRB1\*1201, \*1202 and \*1301: Rare presence of DRB1\*1201-DRB3\*0202 (HF 0.4%), DRB1\*1202-DRB3\*0202 (0.3%), and DRB1\*1301-DRB3\*0202 (0.1%) was observed. These data were compared with the DRB1-DRB3 haplotypes of Black, Caucasian and other Asian populations (11th IHW data): Koreans and other Asian populations show less polymorphism in the distribution of DRB1-DRB3 haplotypes with very high RLD (0.9–1.0) values. DRB1\*0301, \*1201 and \*1301 alleles show different DRB1-DRB3 haplotypic associations among different populations.

**(P1-19) Variation of HLA class II genes in nganasans and kets, two indigenous siberian populations**

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Allelic frequencies at the three most polymorphic loci of the HLA class II region (*DRB1*, *DQA1*, and *DQB1*) were determined in 24 Nganasans and 17 Kets, the remnants of the two most ancient groups of the Lower Yenisey River/Taimyr Peninsula region in northern Siberia. By single-stranded conformational polymorphism typing, verified by sequencing the corresponding segments of genomic DNA, 19 HLA-*DRB1-DQA1-DQB1* haplotypes and 15 HLA-*DRB1*, 7 *DQA1*, and 11 *DQB1* alleles were found. The most frequent alleles were *DRB1\*1301* (23.5%), *DQA1\*0103* (29.4%), *\*0501/03/05* (29.4%), and *DQB1\*0301/0309* (32.4%) in the Kets, and *DRB1\*0901* (25%), *DQA1\*0301* (39.6%), and *DQB1\*0301/0309* (37.5%) in the Nganasans. The distribution patterns and comprehensive phylogenetic analysis based on the haplotype frequencies of 17 Siberian populations suggest that the founders of both Kets and Nganasans came from Paleolithic populations in the Altai-Sayan Upland. The high frequency of the *DRB1\*0901* allele in the Uralic-speaking Nganasans may be the result of a relatively recent gene flow from the northwardly-expanding Tungus-speaking Evenki who originated in the Baikal area.

**(P1-20) HLA class I (-A, -B, -Cw) and class II (-DRB1, -DQA1, -DQB1) Allele and Haplotype Frequencies in the Present-day Thais.**

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HLA class I (-A, -B, -Cw) and class II (-DRB1, -DQA1, -DQB1) allele and haplotype frequencies were determined for 142 Present-day Thais. Typing was performed by using the PCR-SSOP and SSP methods. Allele frequency of more than 10% were shown:-

HLA Class I: The number of different alleles detected were 15, 22 and 10 for HLA-A, -B and -Cw, respectively.

HLA-A\*:- A\*02, A\*11, A\*24, A\*33

HLA-B\*:- B\*15, B\*40

HLA-C\*:- Cw\*01, Cw\*03, Cw\*04, Cw\*07, Cw\*08

HLA-A\*02 was highly polymorphic with different sub-alleles detected (5) follow by HLA-A\*24 (3) and A\*11(2). Among HLA-B, the highest diverse allele was HLA-B\*15 with 8 sub-alleles were found. HLA-B\*40 was also polymorphic but was unable to give definitely defined sub-allele.

HLA Class II: The number of different alleles were:- 13, 6, 5 for HLA-DRB1, HLA-DQA1, HLA-DQB1, respectively. HLA-DRB1\*14 was highly polymorphic with 5 sub-alleles follow by 4 sub-alleles for HLA-DRB1\*04 and DR2.

For three locus haplotype: the common HLA-A -B -Cw and HLA-DRB1 -DQA1 -DQB1 haplotype (> 5%) were:

HLA-A\*33, B\*58, Cw\*0302

HLA-A\*02, B\*46, Cw\*0102

HLA-DRB1\*1202, DQA1\*0601, DQB1\*0301

HLA-DRB1\*0901, DQA1\*0302, DQB1\*03032

HLA-DRB1\*1502, DQA1\*0101, DQB1\*0501 (There were more than 10 distinct DRB1\*02, DQA1, DQB1 haplotypes.)

For five locus association: HLA-A -B -Cw -DRB1 and -DQB1, 14 haplotypes were found more than 1%. The most three common haplotypes were HLA-A\*33, B\*5801, Cw\*0302, DRB1\*0301, DQB1\*0201; HLA-A\*1101, B\*1502, Cw\*0801, DRB1\*1202, DQB1\*0301 and HLA-A\*0207, B\*4601, Cw\*0102, DRB1\*0901, DQB1\*03032. These haplotypes were also found in other Asian population.

### **(P1-21) Comparative analysis of the HLA DRB1 and DQB1 polymorphism of Vietnamese Kinh and Muong ethnies**

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Blood samples from 103 unrelated Kinh and 83 Muong volunteer donors were collected and typed for the DRB1 and DQB1 loci.

The most frequent DRB1 alleles of the Kinh were 1202 (30%), 0901 (14.2%), 1502 (7.8%) DQB1\*0301 (37%), 0303 (17%), 0501 (11.5%) and haplotypes 1202/0301 (29.7%), 0901/0303 (14.1%), 1502/0501 (6%); while of the Muong DRB1 alleles were 1602 (19.8%), 1402 (15%), 0301 (12.6%), 1502 (12.6%), DQB1 0502 (48%), 0201 (10.9%), 0501 (10.9%) and the haplotypes 1602/0502 (18.7%), 1402/0502 (14%), 0301/0201 (9.7%)

No deviation from expected Hardy-Weinberg equilibrium proportions

Marked differences were observed between DRB1 and DQB1 allele and haplotypes distributions of the Muong with the Kinh, namely: DRB1\*1601 and 1602 ( $P_c = 10 - 5$ ), 1202 ( $P_c = 10 - 4$ ), 1401, 1404 and 1405 ( $P_c = 10 - 3$ ), and DQB1 0301, 0302 and 0303 ( $P_c = 10 - 10$ ); 1202/0301 ( $P_c = 10 - 8$ ), 1401/0502 ( $P_c = 10 - 6$ ), 1602/0502 ( $P_c = 10 - 8$ ). Some were seen only in each ethny: (0818/0301, 1202/0502 in the Muong and 1201/0301 in the Kinh)

A nonsignificant positive normalized deviate of Homozygosity was observed. These values are suggestive of the weak influence of directional and balancing selection on these loci, they are also consistent with a pattern of neutral evolution.

The genetic distance of Arnold for several South-East Asian populations suggests that the Kinh and the Muong were closer for DQB1 and the Kinh and the Thai closer for DRB1. So the question of the common origin must be dealt with additional detailed investigations.

### **(P1-22) HLA polymorphism in Baloch ethnic group from Iran and their genetic relationship to Baloch from Pakistan.**

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The extreme polymorphism in different loci of the HLA system has been used as an invaluable tool for anthropological studies. Determination of HLA allele and haplotype frequencies in different ethnic groups is useful for population genetic analyses and the study of genetic relationships among them.

In the present study, molecular analysis of HLA-A, -B, -C, -DRB1, -DQA1 and -DQB1 has been used for the first time to assign specific HLA allele and haplotype frequencies in the Baloch ethnic group of Iran and compared with the frequencies in Baloch and other ethnic groups of Pakistan.

The results of this study showed that the most frequent HLA class I alleles were A\*02011 (18%), B\*4006 (9%), and C\*04011 (27%). The most common HLA class II alleles were DQA1\*0101/2 (42.5%), DQB1\*0201 (32%), and DRB1\*0301 (29%).

Three-loci haplotype analysis revealed that, A\*3303-B\*5801-C\*0302 with a haplotype frequency of 4% and DQA1\*0501-DQB1\*02101-DRB1\*0301 with a haplotype frequency of 19.6% were the most common HLA-I and -II haplotypes, respectively.

Phylogenetic tree based on DA genetic distances for HLA-A, DRB1, and DQB1 loci showed that, Baloch from Iran are very close to Brahui and Baloch of Pakistan. It may reflect an admixture of Brahui and Baloch ethnic groups of Pakistan in Balochistan province of Iran.

### (P1-23) Human leukocyte antigens class II (DRB, DQA1 & DQB1) alleles frequencies and haplotype association in Iranian normal population

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Objective: The polymorphism of the HLA class II genes DRB, DQA1 and DQB1 was investigated in 100 unrelated healthy Iranian individuals.

Method: 5 ml of 5% whole blood were collected from the healthy blood donor and the genomic DNA was extracted by salting out method. HLA typing was performed using high resolution PCR-SSP technique to determine allele and haplotype frequencies.

Results: The most common DRB alleles were DRB1\*11, DRB1\*15 and DRB1\*0101 with a frequency of 25%, 14.3% and 11.3% respectively. In contrast the allelic frequency of DRB1\*16 and DRB1\*09 was very low (1.5% and 0.7% respectively). The most frequent DQB1 alleles were DQB1\*03011 (24%) and DQB1\*0201 (17.8%). The most frequent DQA1 alleles were DQA1\*0505 (21.2%) and DQA1\*0103 (14%) and the most common haplotype was DRB1\*11-DQB1\*0301-DQA1\*0505 (25%).

Conclusion: The present study suggest that Iranian Caucasian with some degree of differences share some of the HLA alleles with south and eastern European population and these data with our previous study from Fars region of Iran does not show any significant differences.

### (P1-24) HLA in andean aymaras from Peru

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Although Amerindian groups represent a large percentage of the total population of Peru, data on the genetic background of Peruvian Andean population is still scarce. No previous HLA study has focused on Peruvian Amerindian ethnic groups, being this the first report of HLA in Peruvian Andean Aymaras.

Using Reverse Line Blot Assay, HLA typing for Class I (HLA-A, HLA-B, HLA-Cw) as well as Class II (HLA-DRB1 and HLA-DQB1) alleles was done on cryopreserved PBL DNA obtained from 31 Aymaras inhabiting two rural localities in the Peruvian Andes.

Major HLA class I alleles found were HLA-A\*02, HLA-A\*24, HLA-B\*3505/17/30, and HLA-Cw\*0401/05/08. Major HLA class II alleles found were HLA-DQB1\*0303, HLA-DQB1\*0302, HLA-DQB1\*0402, HLA-DRB1\*0404/08, HLA-DRB1\*0802/04/07/11, and HLA-DRB1\*09012.

All alleles found in our study subjects have been previously found in genetically isolated Amerindian populations of South America, and in Peruvian Mestizos.

Native American populations have a limited diversity of HLA alleles, compared with other ethnic groups which reflects a population bottleneck in the founding of these groups.

As the MHC contribute to the genetic susceptibility to autoimmune, cancer, metabolic and infectious diseases, our findings may help identifying groups at risk for certain medical conditions among Andean populations, as well as regarding the efficacies of modern forms of immunization by means of DNA vaccines.

### (P1-25) Diversity of HLA alleles and haplotypes in the Australian population.

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Routine tissue typing of bone marrow transplant patients and donors has resulted in the identification of over 55 new HLA alleles so far in this laboratory (including 5 A, 19 B, 5 C, 19 DRB1 and 4 DRB3 alleles). Analysis of DRB1 allelic results from bone marrow transplant patients, unrelated donors and cord blood donor samples revealed a high level of diversity with nearly 25% of all known DRB1 alleles observed. Sixteen DRB1 alleles, including two novel alleles, were present solely in the patient group, showing the difficulty in finding allelic matched donors for some patients. Within an HLA-DR haplotype, the DRB1 gene appears to always be present and depending on its allelic variant it may be associated with either a DRB3, DRB4 or DRB5 gene or it may lack the second functional DRB gene. With an ever growing number of unique HLA alleles these haplotypic associations are becoming more diverse. Over 6000 Australian high resolution DR haplotypes have been analysed by sequencing based typing in the last three years and over 120 different DR haplotypes identified. Six predominant haplotypes accounted for nearly 50% of all observed haplotypes in this Australian population sample. The majority of the remaining haplotypes were observed at low frequencies with several unusual haplotypes identified.

**(P1-26) HLA variation in peptide binding domains through sequence based typing**

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MHC is a large multigene family involved in the humoral and T-cell mediated immune responses of vertebrates and plays an important role in immune recognition. Because of the critical role of MHC in immune recognition and defense, it would be useful to examine polymorphisms at MHC loci in endangered tribal populations of India. A study was undertaken to analyze genetic variation in exon-2 and exon-3 of MHC loci (which code for antigen recognition regions of MHC). Using specific primers designed for these two domains, genomic DNA from tribal and non-tribal samples from India, were amplified and the amplicon from each individual was cloned in a blunt end cloning vector and sequenced. Sequencing revealed abundant polymorphism not only between the clones of different individuals, but also between the clones of the same individual. In addition many new alleles, hitherto unreported, were also observed. In another part of the study, the HLA genes were amplified using primers of different sub-classes of class-I and the amplicons were sequenced using internal primers. This study also revealed hitherto unreported alleles in tribal populations. Since such polymorphisms can provide vital clues about the complexity in the antigen-binding domain of MHC, the DNA sequence data was converted to putative amino acid sequences using Swiss-Prot software and was studied in detail. It was observed that many of the sequence polymorphisms translated in significant changes in the amino acids in the domain involved in Antigen-MHC interaction. The results of this investigation will be discussed in detail.

**(P1-27) The role of Toll-like receptor 2 polymorphism (TLR2 Arg677Trp) in lepromatous leprosy**

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TLR2 is critical in the immune response to mycobacterial infections and the mutations in the TLR2 have been shown to confer the susceptibility to severe infection with mycobacteria. To define this, we screened the intracellular domain of TLR2 in 96 leprosy patients. Ten subjects among lepromatous leprosy (LL) patients had a band variant detected by SSCP. DNA sequencing detected a C to T substitution at nucleotide 2029 from the start codon of the TLR2. We also investigated the profiles of cytokine, such as IL-10, IL-12 and TNF- $\alpha$  in response to *M. leprae* in mononuclear cells from leprosy patients with the TLR2 mutation (TLR2 Arg677Trp). In leprosy patients with TLR2 mutation, production of IL-12, as well as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), by *M. leprae*-stimulated monocytes were significantly decreased compared with that in normal healthy with TLR2 wild-type. There was no significant difference in gamma interferon (IFN- $\gamma$ ) production between mutant and wild-type during early stimulation (18 hr). However, the cells from patients with the TLR2 mutation showed significantly increased production of IL-10 after 18 hr of stimulation.

Our results provide the first genetic evidence that mutation in TLR2 is associated with leprosy and evidence that depressed IL-12 and TNF- $\alpha$  in response to *M. leprae* is involved in the immunopathogenesis of leprosy patients with TLR2 mutations (TLR2 Arg677Trp).

**(P1-28) Association studies of KIR gene haplotype with the susceptibility to rheumatoid arthritis or pollinosis**Akiko Ishitani<sup>1</sup>, Takahiro Yamashita<sup>2</sup>, Norikazu Murata<sup>3</sup>, Yoko Kameoka<sup>1</sup>, Mari Nakanishi<sup>1</sup>, Tsuneo Ashida<sup>4</sup>, Takeshi Ide<sup>5</sup>, Kiyoe Masuo<sup>6</sup>, Masaharu Sada<sup>7</sup>, Katsuhiko Hatake<sup>1</sup>, Daniel Edward Geraghty<sup>8</sup><sup>1</sup>Nara Medical University, <sup>2</sup>University of Tokyo, Dep. of Obs. and gyn., <sup>3</sup>Kyowakai Hospital, Center of Rheumatism, <sup>4</sup>Ashida ENT Clinic,<sup>5</sup>Nara Medical University, Dep. of Chemistry, <sup>6</sup>Veritas Corporation, <sup>7</sup>National Cardiovascular Center, <sup>8</sup>Fred Hutchinson Cancer Research Center

Natural killer immunoglobulin-like receptors (KIRs) interact with HLA class I molecules and regulate natural killer cells, thus playing an important role in the non-adaptive immune response. The KIR gene family lies in a tandem array on a genomic subregion on chromosome 19q13.4, with distinct haplotypes made up from a subset of some 18 distinct KIR genes and pseudogenes. We have developed methods to type KIR genes using sequence specific primers (SSP) and to determine haplotype content based on a gene-profile from several haplotypes analyzed by sequencing or family studies. Using this method we studied the association of the KIR genes profiles or haplotypes with the susceptibility to rheumatoid arthritis (RA) or pollinosis. Blood samples from 94 patients with rheumatoid arthritis, 177 with Japanese cedar pollen allergy and 81 without any symptoms of RA and pollinosis were used. The frequency of the gene profiles and haplotypes of patients and controls were analyzed for association with disease. We failed to find any association of KIR gene with rheumatoid arthritis or pollinosis. However, the data of frequencies of KIR gene profile and haplotypes in Japanese may be useful for other association studies.

**(P1-29) Comparative analysis of the NFBD1 gene by genomic sequencing: species-specific structural feature**

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The NFBD1 (a nuclear factor with BRCT domains protein 1)/KIAA0170 gene mapped to the central part of the HLA class I region, encoding a nuclear protein containing an FHA motif at its N-terminus, two BRCT motifs at its C-terminus, and internal repetitions of a 41 amino acid. These motifs were highly conserved in all the examined species. We identified that the NFBD1 gene resides at the orthologous positions of the human, chimpanzee, rhesus macaque, pig, mouse, and rat MHC class I regions by comparative sequencing analysis. The most conspicuous feature in this gene is that the numbers of internal repetitions are variable among species. So far, we have found 13 amino acid repeats in human and pig (although a small deletion was recognized in pig), 15 repeats in chimpanzee and rhesus macaque, and 8 repeats in mouse and rat. However, it must be noted that no genetic polymorphism or no difference in the number of internal repetitions has been recognized in 100 human genome DNA samples and several chimpanzee cell lines, suggesting that the number of internal repetitions was highly conserved in each species. NFBD1 was a mediator of the mammalian DNA damage checkpoint, so participating in DNA damage response at the early stage. Therefore, the differences in the number of internal repetitions in NFBD1 involved in homeostasis is novel, presumably reflecting functional importance of the physical contact or recognition by several NFBD1 interactive molecules with different structures depending on species, which is now under way in our laboratory.

**(P1-30) DRB1\*04 share epitope alleles and Tumor necrosis factor gene polymorphism (-863) in Thai patients with rheumatoid arthritis**

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Rheumatoid arthritis (RA) in Thai population is significantly associated with HLA-DR4 shared epitope (SE) alleles (DRB1\*0401 and \*0405) similar to many populations. The frequency of DRB1\*0401 and \*0405 was 38% in RA patients and 16% in the healthy controls ( $P = 0.005$ ,  $OR = 3.24$ ,  $95\% CI = 1.38, 7.74$ ). In addition, TNF-863A alleles was analyzed in this study and found to be significantly increased in RA patients compared to controls ( $p = 0.033$ ,  $OR = 2.01$ ,  $95\% CI = 1.05, 3.87$ ). *In vitro* reporter gene analysis has shown that TNF-863A allele associated with increased TNF production, suggesting the role of this polymorphism in susceptibility to rheumatoid arthritis. With respect to disease severity, the DR4 SE and TNF-863A allele did not distinguish patients with more severe disease, as reflected by disease duration, joint deformities, joint erosion and extra-articular features. However, a highly significant association was found between rheumatoid factor positivity and RA patients with DR4 SE ( $p = 0.0005$ ,  $OR = 9.42$ ,  $95\% CI = 2.26-45.27$ ).

**(P1-31) Association of HLA-DRB, DQA1 and DQB1 alleles and haplotypes frequency with pulmonary tuberculosis in Iranian patients.**

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Objective: investigation of HLA-DRB1, DQA1 and DQB1 allelic polymorphism in Iranian pulmonary Tuberculosis and a control group.  
Method: Forty sputum positive pulmonary tuberculosis patients and a group of healthy control consisting of 100 individuals were studied for MHC class II allelic polymorphism by PCR-SSP method. The primer were supplied by biotest in the standard kit, DRB low resolution SSP and DQA, DQB intermediate resolution SSP.

Results: comparison of patients and control group showed a significant increase in frequency of HLA-DRB1\*07 and DQA1\*0101 ( $OR = 2.7$ ,  $95\% CI = 1.97-6.135$ ,  $Pc = 0.025$  and  $OR = 2.66$ ,  $95\% CI = 1.15-6.44$ ,  $Pc = 0.04$  respectively) in contrast the frequency of DQA1\*0301 and DQA1\*0501 were significantly decreased ( $OR = 0.254$ ,  $95\% CI = 0.075-0.865$ ,  $Pc = 0.033$  and  $OR = 0.53$ ,  $95\% CI = 0.04-0.95$ ,  $Pc = 0.045$  respectively). Concerning the haplotypes frequency, DRB1\*1501, QDQA1\*0103, DQB1\*0601 was increased but statistically not significant. In the DQB1 locus, DQB1\*0501 was insignificantly over presented in our study.

Conclusion: these data suggesting that HLA-DRB1\*07 and HLA-DQA1\*0101 are the predisposing alleles and HLA-DQA1\*0301 and 0501 are the protective alleles in our pulmonary Tuberculosis patients.

### **(P1-32) HLA-DRB1, DQB1 polymorphism and G6PD deficiency of muong ethny in hoa binh - Viet Nam**

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G6PD is known as one of the most common hereditary diseases. A possible link with the HLA complex was put forward as this study subject on the Muong ethny of Hoa Binh province where malaria had been endemic with frequent severe form.

Materials and Methods. 91 clinically normal and 26 suspected for G6PD deficiency were PCR typing for HLA-DRB1 and DQB1 by PCR-SSO. The suspected were assayed qualitatively, quantitatively for G6PD deficiency and DNA analysed for variants by MPTP after Shirakawa & al.

Results:

1. The Muong had a high frequency of HLA-DRB1\*1602 (20%), \*1401 (15%), \*0301 (12,6%); HLA-DQB1\* 0502 (48%), \* 0201 (10,9%), \*0501 (10,9%) and haplotypes HLA-DRB1/HLA-DQB1 \*1602/0502 (19%), 1401/0502 (14%) quite different with their neighbours Kinh whose G6PD deficiency was also lower (1%)

2. There was no association between these HLA class II antigens and the disease. The risk ratio was only 1,08 for the most frequent allele DQB1\*0502

3. They had also a high prevalence of G6PD deficiency (28%) and five G6PD molecular variants were detected of them the Union was predominant (44%) quite different with the Malaysian whose Viangchan and Mediterranean variants were the most frequent. The most interesting constatation was 87,5% of this variant went with HLA-DQB1\* 0502 allele

Further investigations were needed

### **(P1-33) DPB1\*0301 and DRB1\*0901: independant markers for aspirin-intolerant asthma in Koreans**

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A possible involvement of immune mechanisms associated with several HLA genotypes has been suggested in Caucasian patients with aspirin-intolerant asthma (AIA). This study aimed to evaluate HLA associations in aspirin-intolerant asthma compared to aspirin-tolerant asthma and normal controls in the Korean population. Seventy-six aspirin-intolerant asthmatics showing positive results on lysine-aspirin bronchoprovocation test, 73 aspirin-tolerant asthmatics and 91 normal healthy controls were enrolled. HLA-DRB1, DQB1, and DPB1 genotypings were performed by direct DNA sequencing analysis using ABI-3100 automated DNA sequence analyzer. The frequencies of DPB1\*0301 and DRB1\*0901 in aspirin-intolerant asthma (13.8% and 18.4%) were significantly higher than that of aspirin-tolerant asthma (4.1% and 9.6%) and normal controls (2.2% and 9.4%) ( $p = 0.004$  and  $0.028$ ). The  $p$  values in DPB1\*0301 association remained significant when corrected for multiple comparisons. The frequencies of two-locus haplotypes (HLA-DRB1-DQB1) in the AIA group showed DRB1\*0901-DQB1\*03032 in aspirin-intolerant asthma (16.9%) were significantly higher than that of aspirin-tolerant asthma (6.0%) and normal controls (7.4%). These findings suggest that HLA-DPB1\*0301 and DRB1\*0901 could be independent markers for the development of aspirin-intolerant asthma in the Korean population.

### **(P1-34) HLA Class II (DRB1, DQA1 and DQB1) allele and haplotye frequencies among HIV-Infected discordant couples in thais.**

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We investigated the association of HLA-DRB1, DQA1 and DQB1 alleles and haplotypes in 33 Thai women with discordant HIV-1 status (22 wife only infected and 11 husband only infected) and their heterosexual seronegative partners. A significant decrease in the frequency of DRB1\*14 was found among the seropositive discordant individuals compared with the HIV-negative controls (3.0% vs 11.3%,  $p = 0.048$ , respectively). DQA1\*0103 were also slightly decreased (0.0% vs 5.6%,  $p = 0.042$ ). The haplotype analysis revealed that DRB1\*1602-DQA1\*0101-DQB1\*0502 (4.6 % vs 0.0%,  $p = 0.024$ ) was significantly increased associated with HIV-1 infection but DRB1\*1502-DQA1\*0101-DQB1\*0501 (1.5% vs 8.1%,  $p = 0.049$ ) decreased. On the other hand, DRB1\*1501-DQA1\*0102-DQB1\*0601 was the most highly associated with HIV-1 uninfected individuals (7.6% vs 0.0%,  $p = 0.002$ ) followed by DRB1\*0405-DQA1\*0302-

DQB1\*0401 and DRB1\*1401-DQA1\*0104-DQB1\*05031 (7.6% vs 1.3%,  $p = 0.024$  and 6.1% vs 0.0%,  $p = 0.007$ , respectively).

### **(P1-35) Characterization of mushroom allergy by facing on two approaches**

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We previously reported that two-thirds of workers in a mushroom plant cultivating *Hypsizigus marumoreus* (Bunashimeji) complained of respiratory symptoms, and a half of those revealed a bronchial hyper-responsiveness that was shown the increase in Th2 type T cells, Th2/Th1 ratio and serum IL-13 and the decrease serum IFN- $\gamma$  as compared with healthy controls. Therefore it is suspected that asthma-like illness might be induced by exposure to the mushroom spores. Interestingly, since these symptoms disappeared when they were exposed by other mushrooms spores, such as *Pleurotus eringii* (Eringi) and *Grifola frondosa* (Maitake), it seemed to be specific dependent on the mushroom species. To address these immunological backgrounds, we are trying to do with two approaches. One investigates whether there is relationship between mushroom allergy and HLA class II alleles of DPB1, DQB1, and DRB1 by using the PCR-RFLP method. The other is to identify these allergic components of *Hypsizigus marmoreus* spore. We already have detected some antigens spot on 2D-PAGE that is detectable by patient's serum. In this symposium, we will discuss these points.

### **(P1-36) Age-related association of HLA-DRB1 \*0101 with type 2 diabetic nephropathy in Japanese patients on maintenance hemodialysis**

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Diabetic nephropathy is a serious microvascular complication in patients with type 2 diabetes as well as type 1 diabetes. However, not all patients with long-term diabetes develop nephropathy. Long-term observational studies have shown that at most only 35% of diabetic patients develop nephropathy after 15–18 years of the disease, irrespective of glycemic control. The underlying mechanisms for the susceptibility of some but not all diabetic patients to nephropathy remain unresolved. Several studies have suggested that diabetic patients prone to nephropathy are genetically susceptible to this complication. The aim of this study was to investigate HLA-DRB1-associated susceptibility to type 2 diabetic nephropathy on maintenance hemodialysis. We recruited 75 patients with type 2 diabetic nephropathy who were treated by hemodialysis. HLA-DRB1 alleles were assigned using polymerase chain reaction followed by hybridization with sequence-specific oligonucleotide probe and restriction fragment length polymorphism methods. As control, data of 493 healthy Japanese controls were taken from Eleventh International Histocompatibility Workshop. A significantly higher frequency of HLA-DRB1\*0101 allele was found in patients compared to the control (18.7% vs 9.7%,  $P = 0.02$ ). None of the other allele tested was significantly more or less frequent in patients than in controls. When stratified by age-of-onset, the results showed the frequency of DRB1\*0101 was significantly higher in patients with early-onset diabetes than in those with late-onset disease (38.1% vs 11.3%,  $P = 0.008$ ). Our findings suggest that DRB1\*0101 is a genetic marker that is strongly associated with early-onset type 2 diabetic nephropathy in Japanese patients.

### **(P1-37) The difference of the immunoresponse to varicella zoster virus (VZV) and HLA association**

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Varicella-zoster virus (VZV) causes both varicella and herpes zoster (HZ). Varicella is the primary infection with a viraemic stage, after which the virus persists in nerve ganglion cells. HZ is the result of reactivation of this latent virus. In some HZ patients who have severe pain persistency more than three months after healing eruptions give a definition as post herpetic neuralgia (PHN). But, HZ is not observed in the patients of varicella and PHN is observed in to all the patients of HZ. This fact is conjectured to be generated according to the difference in an immunoresponse of the individuals to VZV. We have analyzed polymorphisms of HLA genes and 11 sets of microsatellite markers located in the HLA lesion to investigate genes related to PHN and HZ. A total of 214 Japanese patients (102 with PHN, 60 without

PHN and 52 controls) were genotyped by STB method for the class I loci, and PCR-RFLP method for the class II loci. We recognized positive associations of the development of PHN with HLA-A\*3303-B\*4003-DRB1\*1302 haplotype, and negative associations of the development of PHN with HLA-B\*4001 and negative associations of the development of HZ with HLA-B\*5101. These associations were supported by the results of microsatellite analysis. So, these findings suggest that the genes related to response for VZV might exist in the HLA class I region rather than class II.

### **(P1-38) Genetics of KIR and HLA molecules and psoriasis**

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It has been well known that psoriasis vulgaris (PV) is associated with HLA class I antigens, especially HLA-Cw6, and is a chronic inflammatory skin disease via mainly immunocytes. Out of these immunocytes, recently NKT cells and some CD8+ T cells are considered to be more important in PV, and their activities are modified by several HLA-restricted molecules, namely Killer Ig-like Receptors (KIR). In order to investigate genetic relationships between KIR and HLA class I antigens in PV, 96 Japanese PV patients and 50 normal controls were collected. DNA typing of KIR was performed by a method of PCR-SSP.

The data showed that the frequency of activating KIR2DS1 was significantly higher in the PV patient group than in controls (43/96 (44.8%) vs. 14/50 (28.0%),  $p < 0.05$ ), and also there was some absence of inhibitory KIR2DL1 in the PV patient group, but not in controls (7/96 (7.3%) vs. 0/50 (0%),  $p > 0.05$ , n. s.). Interestingly both of these two types of KIR are the molecules which can recognize HLA-Cw6. The subjects with activating KIR2DS1 are supposed to be susceptible to PV, and this activating KIR may play a key role in the activation of pathogenic NKT cells and some CD8+ T cells. The absence of inhibitory KIR2DL1 may also influence on this activation. The present data may lead to a new insight on the molecular basis concerning the pathogenesis of PV.

### **(P1-39) Genomic diversity of MHC as a hallmark in HIV vaccination approaches**

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MHC genes have been suggested to influence disease progression following HIV infection. Allelic variants of the HLA molecules can bind and display various HIV derived peptides with differing affinities to CTLs thereby influencing the efficiency of immune protection. Therefore, defining optimal restriction elements and relevant HIV epitopes conserved across global clades is a hallmark necessity of MHC based vaccine design. And this in turn, is limited by the extreme molecular diversity of MHC as well as hypervariability in HIV in different geographical populations. This study compares distribution patterns of molecular subtypes of HLA class I and class II extended haplotypes in India with reference to global distribution. In Caucasian populations, the A1-B8-DR3 haplotype (AH8.1) is at distinct biologic disadvantage as this is associated with fast progression to AIDS and also with a dysfunctional immune response and autoimmunity. In the Asian Indians, however, this haplotype occurs rarely and has been replaced by another independently evolved AH8.2, i.e. HLA-A26, -B8, -DR3. Its role in influencing HIV susceptibility in this population has been investigated. This observation alongwith (i) high preponderance of HLA-B\*35Px alleles implicated in inefficient immune response and fast progression, (ii) low occurrence of a non-functional mutant allele of CCR5 (CCR5D32), and others in the Indian gene pool imply an immunogenetic basis for possible predisposition and fast progression of HIV infection in India.

### **(P1-40) Peptide carrying DQ6 motifs from human papilloma virus type 16 E7 protein with motif for HLA-DQ6 (B\*0602) shows good TH2 cytokine response.**

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Carcinoma cervix is associated with Human Papilloma Virus (HPV). DQ6 (DQB1\*0602) is positively associated with Cervical Intraepithelial Neoplasia (CIN), HPV16 positive CIN and Cervical Cancer in women from Northern Sweden. The aim of the study was to use peptides identified from screening HPV-E7 protein sequence using motifs from DQ6 molecule. These motifs were previously identified by peptide elution from DQ6 and peptide sequencing. Several peptides were identified. Eight of them were used for the T cell studies. Lymphocytes from women with HPV 16 infection who have cleared the infection compared to those who have not cleared the infection at one-year follow-up were studied.

Lymphocytes from two women were incubated (with and without Brefeldin) with peptides in four sets of experiments with 2 peptides pooled in each experiment. Monoclonal antibodies to CD8, CD4, CD69, TNF $\alpha$  and IFN $\gamma$  were used for further incubation. The cells and

the cytokines were subjected to 4-colour FACS analysis. The results showed, 4 of the 8 peptides were giving high IFN $\gamma$  secretion. Of the 4 peptides, one pool containing 2 peptides gave higher IFN $\gamma$  secretion from CD8 positive cells.

In conclusion, some of the peptides carrying DQ6 motifs from HPV-E7 protein produced IFN $\gamma$  secretion from CD8 positive cells when incubated with lymphocytes from women infected with HPV16. These interesting results might be useful for future prediction strategies for CIN and invasive cancer and for subsequent planning for intervention strategies.

#### **(P1-41) Analysis of CD4/CD8 T cells and related cytokines in chronic rejection of kidney allografts.**

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To characterize the T-cells mediating chronic rejection (CR) of human kidney allografts, we performed RT-PCR analysis of mRNA for CD4, CD8, IFN-g, IL-4, and IL-10 in biopsy specimens. The mRNA expression were compared among following pathological groups: chronic allograft nephropathy (CAN: 8 cases), FK506/CyA-associated nephrotoxicity (Ntox: 6 cases), CAN + Ntox: 8 cases, no rejection (NR: 6 cases), and 1 hour biopsy (1h: 16 cases) as the negative control. These groups exhibited the following relation in CD4 expression: (CAN) = (CAN + Ntox) > (Ntox) = (NR) = (1h). The CD8 expression was (CAN) = (CAN + Ntox) = (Ntox) > (NR) >= (1h). In the cytokine analysis, the relation of IFN-g expression was (CAN) = (CAN + Ntox) >= (Ntox) > (1h). IL-4 was detected in one case each of CAN, CAN + Ntox, and CAN + Ntox + AR (acute rejection), whereas IL-10 was detected in two cases of CAN. These results suggested that CD4 T cells and IFN-g (Th1 response) contribute to most of CAN, and IL-4 and IL10 (Th2 response) to some CAN. On the other hand, these immunological factors exhibited the lesser extent of contribution in Ntox formation.

#### **(P1-43) The regulatory role of p53 family members in thymic epithelial cells**

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The thymus is the primary lymphoid organ that provides an optimal milieu for the development of a functional T cell repertoire via major histocompatibility complex (MHC)-based selection. This inductive environment is constituted by various stromal cells, including thymic epithelial cells (TECs), which compose largely stationary stromal cells. TECs make the three-dimensional framework within which all other thymic cell types reside, although regulatory mechanisms of TECs are mostly unknown. In this study, we investigated the functional significance of p53 family members such as p63 and p73 in human TECs because they showed epitheliotropism in other tissues. The results demonstrated a particular distribution pattern of p53 family members in thymic epithelium, in which cortical TECs preferentially expressed p63 in their nuclei whereas subcapsular / perivascular and medullary TECs displayed nuclear localization of both p63 and p73. More interestingly, it was found that p63 regulated the expression of intercellular adhesion molecule-1 (ICAM-1) and HLA-DR by in vitro studies using human TEC lines. TEC lines transfected with p73 showed the enhancement of the secretion of various cytokines including GM-CSF and M-CSF. This would probably explain the fact that dendritic cells and other cells are localized mainly around subcapsular / perivascular and medullary TEC in human thymus. These suggest that p63 and p73 of p53-related transcription factors are actively involved in preserving thymic microenvironment in their specified regions.

#### **(P1-44) Identification of SH2D1A mutation in a hypogammaglobulinemic male diagnosed as having common variable immunodeficiency**

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Common variable immunodeficiency (CVID) is a highly heterogeneous disease with an unpredictable pattern. CVID appears to have a similar immunological and clinical phenotype to some hereditary humoral immunodeficiency, including X-linked lymphoproliferative disease (XLP). The differential diagnosis between CVID and XLP is clinically important, since they have markedly different prognoses and treatment. The recent identification of XLP gene, known as SH2D1A, has permitted definitive diagnosis of XLP. In this report, we describe a male patient with XLP, who was initially diagnosed as having CVID and developed a fatal course. Using genetic analysis, he was confirmed to harbor the SH2D1A gene mutation. The results support the notion that the possibilities of SH2D1A gene mutation

should be considered in hypogammaglobulinemic male before diagnosis of CVID is made.

### (P2-1) Is the frequency of novel alleles arising as the result of a single nucleotide polymorphism being underestimated?

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Our Department has used Sequence Based Typing (SBT) for all routine HLA DRB1 typing since 1998 and for all routine HLA -A, -B and -C typing since 2002. In that time we have identified 11 novel alleles; 2 at HLAA, 3 at HLAB, 3 at HLAC and 3 at HLA-DRB1 within our local population. These novel alleles have all arisen as the result of a single nucleotide polymorphism (SNP) and are not due to the shuffling of sequence motifs. Five of the 11 SNP's are found within normally non-polymorphic regions of the gene, and these have resulted in amino acid changes. The remaining 6 SNP's are in variable regions of the gene. Two of these, B\*40XX and DRB1\*1509 are unique SNP's to the new allele and have resulted in an amino acid change.

SBT detected all 11 novel alleles identified in our population. A commercially available SSO kit did not detect DRB1\*1509, and it is unlikely that either SSO or SSP methods would detect polymorphisms within conserved regions of the gene.

Our data suggests that SNP's are the most common cause of newly identified alleles, and that by using alternate methods such as SSO or SSP, their frequency is being underestimated.

Locus	Allele	Position	AA change	Conserved or Variable region
HLAA	<sup>1</sup> A*03/11	exon3	no	Conserved
	<sup>1</sup> A*01/02	exon2	yes	Conserved
HLAB	<sup>3</sup> B*0717	exon3	yes	Variable
	<sup>2</sup> B*3924	exon3	yes	Conserved
	<sup>1</sup> B*40XX	exon3	no	Variable
HLAC	<sup>1</sup> C*06XX	exon2	yes	Variable
	<sup>1</sup> C*07XX	exon3	yes	Conserved
	<sup>2</sup> C*0310	exon2	yes	Variable
DRB1	<sup>3</sup> DRB1*1509	exon2	yes	Variable
	<sup>3</sup> DRB1*1434	exon2	yes	Variable
	<sup>1</sup> DRB1*04XX	exon2	no	Conserved

<sup>1</sup> further evaluation in progress before submission to WHO Nomenclature Committee.

<sup>2</sup> novel when identified in our laboratory, but submitted by another group to the WHO Nomenclature Committee.

<sup>3</sup> identified and submitted by DCI to WHO Nomenclature Committee.

### (P2-2) Identification of a “novel” non-HLA antigen in Oriental populations

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In a routine HLA antibody screening cross-match test using the complement-mediated lymphocytotoxicity assay, we discovered an antibody, in a transfused Caucasian woman, recognizing an Oriental restricted antigen that does not appear to be associated with the human leukocyte antigens (HLA) system. The antigen was tentatively named CY (dedicated to the founder of Buddhist Compassion Relief Tzu Chi Foundation, Dharma Master Cheng Yen). CY antigen was found in Chinese, Japanese, Vietnamese and Thai people suggesting that some of these people are closely related. To our knowledge, this is the first confirmed non-HLA antigen restricted to the Oriental ethnicity. The characterization, distribution of this “novel” non-HLA antigen in Taiwanese population and the results of family studies will be presented.

### (P2-3) Genome-wide association study of human narcolepsy using 25,000 microsatellite markers with pooled DNAs

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Narcolepsy is one of a sleep disorder and also a multifactorial disorder. A genetic factor associated with the disorder has been found in the human leukocyte antigen (HLA) region: HLA-DRB1\*1501-DQB1\*0602 haplotype confers strong predisposition to narcolepsy.

We performed the genome-wide association study using about 25,000 microsatellite markers as a tool for searching disease susceptibility regions. The subjects investigated in this study were all Japanese living in the Tokyo area. One hundred and five narcoleptic patients were pooled for the 1<sup>st</sup> set and the other 110 samples were pooled for the 2<sup>nd</sup> set. Similarly, 210 unrelated healthy individuals as controls were pooled for the 1<sup>st</sup> set and the other 210 samples were pooled for the 2<sup>nd</sup> set. We used the 1<sup>st</sup> set pooled DNA for the primary screening of microsatellite markers and the 2<sup>nd</sup> set for the second screening to avoid false positive associations. Allele frequencies were estimated from heights of the peaks detected by an automated sequencer (ABI 3700) with GeneScan software. Then, we performed Fisher's exact test using  $2 \times 2$  or  $2 \times m$  ( $m$ ; allele number) tables. After the analysis, five markers showed the significant differences in both screenings ( $p < 0.001$ ). These markers still showed strong associations with the disorder in the following analysis using all individual samples. Three candidate regions are now subjected to SNP analysis to further narrow down the susceptibility regions of narcolepsy.

### (P2-4) Molecular genetic analyses on human NKG2C (KLRC2) gene deletion

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Human NKG2A, NKG2C and NKG2E genes are located on 12p13 in the natural killer gene complex. We recently identified deletion of NKG2C in a Japanese population. This study was performed to identify the breakpoint and to examine the association of NKG2C deletion with susceptibility to rheumatoid arthritis and systemic lupus erythematosus. By comparing the genomic sequences between NKG2A and NKG2E, the breakpoint was determined to be 1.5–1.8 kb telomeric from the 3' end of NKG2A. Based on this information, a genotyping system was developed. Frequency of NKG2C deletion haplotype was 20.2% in Japanese and 20.0% in Dutch populations. Frequency of homozygous deletion was 4.1% in Japanese and 3.8% in Dutch. Evidence for an association with rheumatic diseases was not detected. These results indicated that NKG2C deletion is commonly present in Japanese and Dutch, suggesting that NKG2C is not essential for survival and reproduction, and is not associated with rheumatic diseases.

### (P2-5) Association of *LIR6* (*LILRA1*) polymorphisms with susceptibility to systemic lupus erythematosus

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LIRs are activating or inhibitory receptors expressed in hematopoietic cells. *LIR6* (*LILRA1*) is an activating receptor expressed on B cells, monocytes/macrophages and dendritic cells and binds to HLA-class I molecules. Although two splicing isoforms exist, functional differences among them are not clear. In this study, we performed a polymorphism screening and also examined for the association with systemic lupus erythematosus (SLE). Direct sequencing of the genomic DNA from 18 Japanese individuals revealed 24 SNPs, including 8 nonsynonymous substitutions, *V5L*, *R12G*, *H164Y*, *S194W*, *L220P*, *R289P*, *Y291H* and *Y301H*. Analysis of 31 Japanese families revealed that these SNPs form two major haplotypes in each of the promoter and extracellular regions, and they showed significant linkage disequilibrium both in the controls and SLE patients (RLD = 0.98 and 1, respectively). Case-control association study was done on 163 SLE patients and 414 controls by direct sequencing or PCR-SSCP, based on the assumption that the haplotypes are conserved in Japanese. Carrier frequencies of one of the promoter haplotypes and one of the extracellular region haplotypes were significantly decreased in SLE ( $P = 0.01$ , OR = 0.63 and  $P = 0.005$ , OR = 0.59, respectively). Significant linkage disequilibrium was not present between *LIR1* (*ILT2*, *LILRB1*) and *LIR6* haplotypes. These results suggested that the polymorphism of *LIR6* is associated with susceptibility to SLE in Japanese through changes in the expression level and/or the binding activity with HLA-class I.

### (P2-6) Association of leukocyte immunoglobulin-like receptor 1 (LIR1, ILT2, LILRB1) polymorphism with susceptibility to rheumatoid arthritis (RA) in Japanese

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LIR1 is an inhibitory receptor broadly expressed in hematopoietic cells, and recognizes HLA-class I and cytomegalovirus UL18. In this study, we considered *LIR1* as a candidate susceptibility gene to RA, and performed a polymorphism screening and association analysis. Direct sequencing of the genomic DNA from 18 Japanese individuals revealed 17 SNPs, including 5 nonsynonymous substitutions, *P68L*, *A93T*, *I142T*, *S155I* and *E625K*. Analysis of 31 Japanese families showed 3 major haplotypes of these SNPs in Japanese (*LIR1.01*, *.02* and *.03*). We next performed a case-control study using 559 patients with RA and 449 Japanese healthy controls. The association of *LIR1* with total RA did not reach statistical significance; however, when the association was examined in HLA-DRB1 shared epitope (SE) positive and negative groups separately, *LIR1.01/01* diplotype was significantly increased in RA in the SE negative group ( $P = 0.037$ ,  $OR = 1.86$ ). *LIR1* polymorphisms were in linkage disequilibrium with neither *LIR6* nor *LIR5*, closely located on each side of *LIR1*. Since these haplotypes encode amino acid substitutions in the putative ligand binding domains, we examined whether they cause changes in the ligand-binding affinity using BIACORE system. *LIR1.01* product showed lower affinity to HLA-B35 compared with other two haplotype products (Kd; *LIR1.01* = 50  $\mu$ M, *.02* = 22  $\mu$ M, *.03* = 26  $\mu$ M). These results suggested that LIR1 may be associated with susceptibility to RA through insufficient inhibitory signals caused by the lower affinity with HLA-class I.

### (P2-7) Association study on abdominal aortic aneurysm using microsatellite markers located in the HLA region

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Abdominal aortic aneurysms (AAA) are common disease of maturity. Autoimmunity has been proposed to play a role in the pathogenetics of AAA. In the spectrum of autoimmune disorders certain HLA alleles are reported to be associated with the development of disease. We previously reported a linkage of the HLA-A2, -B61 and -DR15 antigens to the susceptibility to AAA in Japan. In the present study we performed association analysis with 19 microsatellite markers (D6S439, TAP1, D6S2444, D6S2443, T16, CAT, DQ-CARII, D6S273, TNF $\alpha$ , TNF $\beta$ , C1-2-A, MICA-TM, MIB, C1-4-1, C1-2-5, C1-3-1, C2-4-4, C3-2-11, D6S276) in the HLA region to refine the susceptible gene involved in the development of AAA. And we also examined the susceptibility of TAP2 gene to AAA. We used genomic DNAs from 49 patients and 248 unrelated Japanese healthy controls for the association study. Positive association to AAA susceptibility was observed in the frequent alleles of each microsatellite marker located in the class I region, although negative association was seen in the class II region. Allele 200 of TAP1 microsatellite located between HLA-DQ and DP loci was significantly negatively associated with AAA ( $OR = 0.15$ ,  $P = 0.003$ ). TAP2\*0101 frequency in patients group was significantly lower than control group (29% vs. 41 %,  $P = 0.03$ ). These results suggest that TAP gene seems to control the development of AAA.

### (P2-8) A susceptibility gene mapping for developing non-obstructive azoospermia by microsatellite markers located in the HLA region.

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Congenital dysfunction in spermatogenesis, referred to as non-obstructive azoospermia, is presumed a result of genomic abnormalities. We considered the importance of the genetic background of non-obstructive azoospermia and reported that HLA A33-B44-DRB\*1302 haplotype is associated with susceptibility to this disease in Japanese men. In present study, to search for more precise region involving in the development of non-obstructive azoospermia, we performed the association analysis with 21 polymorphic microsatellite markers located in the HLA region. We used genomic DNAs from 67 infertile Japanese men with non-obstructive azoospermia and 248 unrelated healthy Japanese donors for association study using microsatellite markers. The results disclosed that microsatellite markers located in the

HLA class I region or the class III region showed no statistically significant association with this disorder, although the HLA A33-B44 alleles showed a significant association. In contrast, some of the microsatellite markers in the HLA class II region and the HLA-DRB1 and -DQB1 loci showed strong associations with non-obstructive azoospermia. These data suggest that the critical region for development of non-obstructive azoospermia is near the HLA-DRB1 and -DQB1 segments in the HLA class II region.

### **(P2-9) Susceptibility gene mapping for autoimmune pancreatitis in the Japanese population.**

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Autoimmune pancreatitis (AIP) is associated with hypergammaglobulinemia, histologic evidence of lymphoplasmacytic inflammation, occasional coexistence of other autoimmune diseases, and a favorable response to glucocorticoid treatment. We have previously reported that this disease is entity characterized by high serum immunoglobulin G4 concentrations and associated with the HLA-DRB1\*0405-DQB1\*0401 haplotype. In the present study, we have performed association analysis to search definite location for susceptibility to AIP and to refine the genetic contribution in AIP development. We examined polymorphisms of 21 micro satellite markers distributed in the HLA region and polymorphisms of 5'-flanking region of the TNFA and IkBL1 genes in susceptibility gene mapping. Genomic DNAs from 40 AIP and 210 unrelated Japanese controls are used for analysis. Detection of polymorphisms in the 5'-flanking region of the TNFA and IkBL1 genes were determined by PCR- SBT and PCR-SSCP methods. Statistical studies of associated alleles on each microsatellite locus showed that the susceptibility segments for AIP in the HLA region is localized only around the HLA-DRB1 and -DQB1 loci. No statistically strong association was observed in the class III and class I regions. There were also no significant differences in the frequency of alleles in the promoter regions of TNFA and IkBL1 genes. These results suggest that DRB1 and DQB1 genes seem to control the development of AIP.

### **(P2-11) A naturally selected dimorphism within the HLA-B44 supertype alters class I structure, peptide repertoire and t cell recognition**

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HLA-B\*4402 and B\*4403 are naturally occurring MHC class I alleles that are both found at a high frequency in all human populations and yet they only differ by one residue on the  $\alpha 2$  helix (B\*4402 Asp156  $\rightarrow$  B\*4403 Leu156). CTL discriminate between HLA-B\*4402 and B\*4403 and these allotypes stimulate strong mutual allogeneic responses reflecting their known barrier to hemopoietic stem cell transplantation. While HLA-B\*4402 and B\*4403 share >95% of their peptide repertoire, B\*4403 presents more unique peptides than B\*4402, consistent with the stronger T-cell alloreactivity observed towards B\*4403 compared with B\*4402. Crystal structures of B\*4402 and B\*4403 show how the polymorphism at position 156 is completely buried and yet alters both the peptide and the heavy chain conformation, relaxing ligand selection by B\*4403 compared with B\*4402. Thus, the polymorphism between HLA-B\*4402 and B\*4403 modifies both peptide repertoire and T cell recognition, and is reflected in the paradoxically powerful alloreactivity that occurs across this 'minimal' mismatch. The findings suggest that these closely related class I genes are maintained in diverse human populations through their differential impact on the selection of peptide ligands and the T cell repertoire.