New Zealand Blood Service
The gift of life

BLOOD ISSUES

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National Tissue Typing Laboratory

The National Tissue Typing Laboratory is based at the NZBS site in Epsom, Auckland and is responsible for tissue typing tests in support of New Zealand's bone marrow and solid organ transplant programs. The Laboratory also carries out testing for disease association markers, for antibodies implicated in transfusion-related reactions, for antibodies against platelet antigens and is responsible for the provision of compatible or matched platelets. In 2005 the laboratory carried out approximately 3,500 DNA extractions, over 5,000 DNA-based tests and more than 10,000 antibody-related tests on the most complex system found in humans (see below). With such a large number of tests a certain amount of automation is required to minimise the chance of error. NZBS has invested significantly in the past 12 months in new automation technology bringing the Tissue Typing Laboratory up to the standard you would expect of an ASHI (American Society for Histocompatibility and Immunogenetics), and IANZ (International Accreditation of New Zealand), accredited laboratory. A small robot is now used for DNA extractions, another one is used for pipetting and two Luminex platforms are now in action carrying out high throughput tissue typing and antibody screens and then the analysis. Skilled technologists are needed to operate this technology and check the results and there has been some recruitment in this area in the last year. The Auckland laboratory now has a full complement of 18 staff.

Role of the Laboratory

The Tissue Typing laboratory's main role is in testing for compatibility between patients, who require an organ or bone marrow transplant, and potential donors. Testing for compatibility may involve matching patient and donor tissue types and it may involve antibody screening or crossmatching to ensure the patient does not have an antibody directed against donor tissue type.

A person's tissue type comprises a set of distinct proteins called Human Leukocyte Antigens (HLA) which are found on the surface of most cells. HLA molecules, or proteins, relevant in transplantation are called HLA-A, -B, -Cw, -DPB1, -DQB1 and –DR although, routinely, HLA-A, -B and DR are typed for compatibility purposes.

The genes expressing HLA are located in a gene-dense area of chromosome 6 called the Major Histocompatibility Complex (MHC), figure 1. Many genes in this region are involved in modulation of the immune system. HLA's primary role is in the adaptive immune system, recognising and presenting foreign peptides to the immune system.

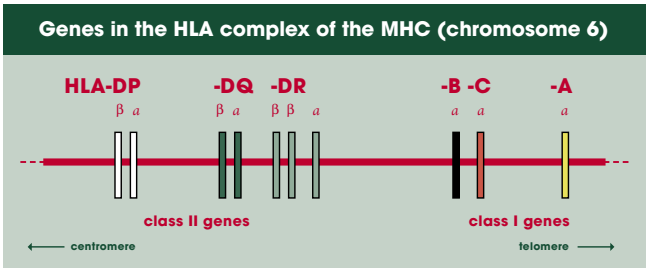

figure 1

HLA Inheritance

Since a person has two copies of chromosome 6, a person will also have two copies of the HLA-A, B, and DR molecules. Each set of HLA antigens is referred to as a "haplotype" and every person inherits one haplotype from each parent. Therefore, a person will share an HLA-A, B, and DR antigen with each of his/her parents, as shown in figure 2.

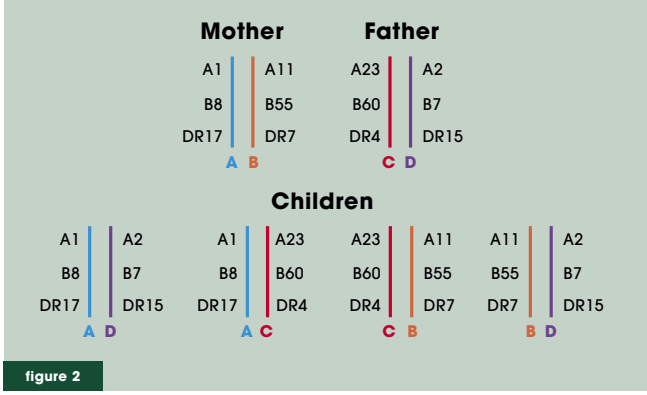

figure 2

HLA Diversity

An important characteristic of the HLA system is the enormous number of HLA types that are possible. Each person has 2 each of the HLA-A, B, and DR antigens. There are roughly 25 different HLA-A antigens, 40 different HLA-B antigens and 20 different HLA-DR antigens. These antigens are expressed by genes and there are many gene variants or alleles – in the case of HLA-B, the most polymorphic HLA molecule, there are over 700 alleles. Therefore, it is not an exaggeration to say that there are potentially millions of possible HLA types in the population.

HLA and Disease

As well as compatibility testing the tissue typing laboratory performs HLA typing for patients to aid diagnosis and treatment of certain diseases. It is well established that susceptibility and/or resistance to certain diseases is primarily associated with genes

encoding peptide-presenting HLA molecules. Type I diabetes, narcolepsy, coeliac disease, ankylosing spondylitis and rheumatoid arthritis have each been clearly associated with specific class I or class II HLA genes. These and other examples are given in the table.

Disease	Associated HLA marker	Relative risk of disease†
Ankylosing spondylitis	B27	87.4
Rheumatoid arthritis	DR 4	4.2
Behçet's syndrome	B51	3.8
Systemic lupus erythematosus	DR 3	5.8
Insulin-dependent (type 1) diabetes mellitus	DR3, DQ2, DR4	3.3
Narcolepsy	DR2, DQ6	5.3
Coeliac disease	DQ2, DQ8	10.8
Multiple sclerosis	DR 2	4.1
Birdshot retinochoroidopathy	A29	109.0

†The relative risk indicates the frequency of a disease in persons with the HLA marker as compared with persons without the marker.

HLA Typing

Historically, HLA types were identified by serology using panels of HLA sera which could identify HLA antigens. Molecular or DNA-based tissue typing has superceded serology especially with the advent of the polymerase chain reaction or PCR. DNA techniques are more efficient and accurate than serology. PCR can specifically amplify HLA genes and different PCR techniques can be used to identify which HLA is present. The main techniques in use in Tissue Typing laboratories are PCR-SSP (sequence-specific primer) and PCR-SSOP (sequence specific oligonucleotide probe).

Luminex

One of the key technologies in use in the Tissue Typing laboratories is Luminex which is a form of flow cytometer. It has many different beads (up to 100) stained with different colours which can be differentiated by the light emitted following excitation by the Luminex laser.

Luminex can be used to identify HLA antibodies in solid organ patients and for HLA typing, figure 3.

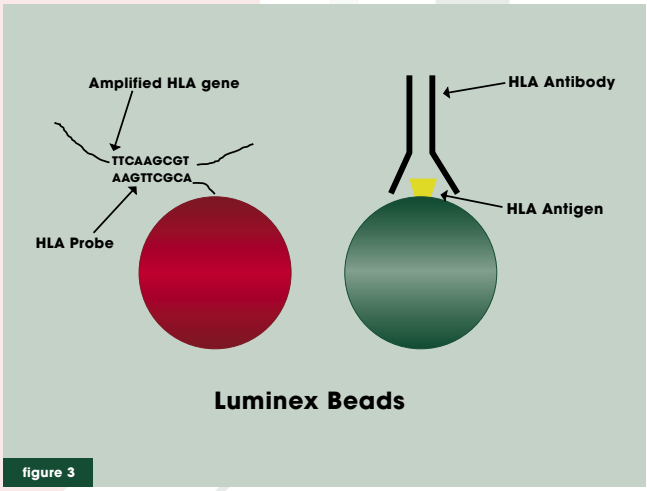

figure 3

HLA and Transplantation

In the case of bone marrow (or haematopoetic stem cell, HSC) transplantation it is important that HLA of patient and donor are matched to minimise graft versus host disease. Large-scale studies on HLA and transplant outcome have demonstrated which HLA loci are critical to match in order to maximise the success of haematopoetic cell transplantation. Although matching three HLA loci traditionally associated with HSC transplantation (HLA-A, -B and –DR) can lead to successful transplantation outcomes, recent research has shown matching at HLA-C can also improve outcome.

In the case of solid organ (i.e. kidney) transplants, when HLA molecules of the donor are different from recipient, the recipient's immune system can make antibodies against the foreign HLA molecules on the transplanted organ. If the immune system is not suppressed, the recipient's immune system may eventually reject the transplanted organ. Today's immunosuppressive drugs have allowed for the successful transplantation of completely HLA mismatched organs. This is because the drugs prevent the recipient's immune system from building a response against the transplanted organ. It is important to note, however, that the better the HLA match, the better the long-term survival of the transplant.

Sensitisation

An individual may make antibodies against foreign HLA molecules if they are exposed to them through pregnancy, transfusion, or transplantation. An individual who has had any of these events is considered "sensitised". For these patients, it is important to first determine if they have any anti-HLA antibodies and if so, what specific HLA molecule(s) they are against. If a patient has antibodies against a specific HLA molecule, any donor that has that antigen expressed on their cells will be considered unsuitable. If a patient is transplanted with a kidney which the patient has preformed antibodies against donor HLA then the patient will experience a hyper-acute rejection of that organ.

Anti-HLA Antibodies

Antibody screening is performed regularly in the laboratory to look for anti-HLA antibodies in the patient's serum. The result of this test is expressed as a Percent/Panel Reactive Antibody or PRA. This value is calculated as the number of cells in a panel that the patient's serum contained antibodies against divided by the total number of cells tested. It is used as a rough estimate of the percentage of donors that a patient will NOT be compatible with. In other words, a patient with a low PRA has a greater chance of finding a compatible donor than a patient with a high PRA.

The laboratory also attempts to identify the specificity of any antibody present. If a patient has a positive PRA (greater than 0%), the laboratory will perform further

testing in order to identify unacceptable antigens, that is, the specific HLA target of the antibodies. Any unacceptable antigens identified for a patient are used to rule out potential donors that will be incompatible by crossmatch, and therefore unsuitable.

Crossmatching

Due to the large degree of diversity in the HLA system, it is not practical for all solid organ transplants to be from HLA matched donors. Instead, transplants must be from HLA compatible donors. This means that the patient does not have antibodies specific to the HLA antigens on the donor's cells. The crossmatch is the last test performed prior to transplantation. The crossmatching techniques used in the HLA laboratory are flow cytometry and complement dependent lymphocytotoxicity (CDC). In both techniques cells from potential donor are mixed with patient serum, any anti-donor HLA antibodies are detected by either cell death (CDC, figure 4), observed under the microscope, or by detection of fluorescence bound to donor cells (flow cytometry). A transplant will only proceed when the recipient has been shown not to have anti-donor HLA IgG antibodies, i.e. negative crossmatch (figure 4).

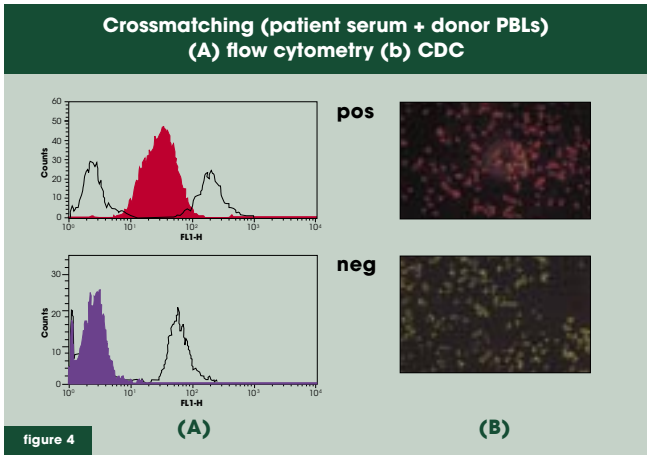

figure 4

Transfusion Related Acute Lung Injury (TRALI)

TRALI is a well-characterised clinical constellation of symptoms including dyspnoea, hypotension and fever. The radiological picture is of bilateral pulmonary infiltrates without evidence of cardiac compromise or fluid overload. Symptoms typically begin 1-2 hours after transfusion and are fully manifested within 1-6 hours. Products typically infiltrated in TRALI are whole blood, packed red blood cells, fresh frozen plasma, cryoprecipitate, platelet concentrates, apheresis platelets and rarely IVIG. The etiology of TRALI may be attributable to the presence of anti-HLA and/or antigranulocyte antibodies in the plasma of multiparous females or donors who have received previous transfusions. TRALI recipients have no specific demographics such as age, gender or previous transfusion history. Although TRALI does not always occur through transfusions from donors with anti-HLA or anti-granulocyte antibodies, one or both of these antibody types have been found in 89% of TRALI cases. The Tissue Typing Laboratory plays an important role in identifying whether these antibodies are present in the donor's serum.

Tissue Typing and Platelets

Some patients have a low platelet count which may be due to a blood disorder or as a side effect of chemotherapy treatment. They require platelet transfusions and the Tissue Typing's role is to check that platelets and patient are compatible or to provide HLA-matched platelets for the patient.

Sometimes a mother can make antibodies against paternal platelet antigens inherited by the unborn foetus. This can cause a reduction in foetal platelet count (a condition called neonatal alloimmune thrombocytopaenia, or NAIT) which may require platelet transfusions for the newly born baby. To understand which antibodies may be produced requires typing of the platelet antigens (called HPA) of the parents and investigation for the presence of NAIT is usually a transient condition but needs to be investigated for future pregnancies. Mothers can also make antibodies against foetus paternal HLA but, as these antibodies do not normally cross the placenta, they do not cause a problem for the foetus.

Tissue Typing Request Forms

With so many tests being carried out in Tissue Typing for different types of patients and donors, and with different specimen requirements for the different tests, three test request forms have been designed and are being distributed by the Transfusion Nurse Specialists.

The sections that are marked * are mandatory and have to be filled in.

Sample requirements, sample acceptance criteria and an address for sending the samples to are listed on each of the forms. All queries about these or test results should be directed to the Tissue Typing Laboratory 09-523-5731.

Sample and Request Form Labelling Errors – Introduction of a NZBS National Database

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New Zealand Blood Service (NZBS) manages the blood banks in six of New Zealand's largest hospitals between them responsible for the major proportion of the country's pretransfusion testing.

The international transfusion literature have shown that labelling errors and misidentification associated

with pretransfusion samples significantly increases the risk of transfusion errors, particularly transfusion of incompatible or 'wrong' blood.

On 1 May 2006 NZBS introduced a national procedure, at its blood banks, for the collection of data regarding errors associated with pretransfusion sample and request form labelling. A list of errors required to be reported has been created based on NZBS sample and request form labelling requirements, in turn based on requirements of the ANZBST 'Guidelines for pretransfusion testing' (2002). The occurrence of these errors is routinely recorded by each blood bank on a national NZBS form and data subsequently entered into a Microsoft Access™ database for analysis and reporting. Additional errors are recorded where this is required by individual hospitals but are not included in the wider analysis of data.

During the period 1 May 2006 to 30 June 2006, 1341 requests with the defined errors in sample and/or request form labelling were received. Of the 1398 specific errors reported 81.9% were due to the five most prevalent errors, namely: patient details - discrepancy between sample and form (20.2%), sample not signed (19.2%), missing/incomplete details (15.7%), sticky label on sample (13.7%) and declaration not signed (13.2%).

The NZBS sample and request form labelling errors database is starting to yield useful data and from this data it is hoped to gain an understanding of the nature and scope of labelling errors seen in the six NZBS blood banks. This knowledge will provide a unique opportunity for raising the awareness of what errors are occurring, awareness which will hopefully precipitate a reduction in numbers.

Malaria Antibody Testing

Malaria is caused by the protozoa of the genus *Plasmodium*, which is transmitted by the bite of the female Anopheles mosquito and can also be transmitted via transfusion of blood infected with the protozoa. There are four species of Plasmodium that cause malaria; *P.falciparum, P.vivax, P.malariae* and *P.ovale. Plasmodium falciparum* is the most serious transfusion risk as infection may prove rapidly fatal. Following infection with plasmodial species, the immune response results in the formation of specific antibodies within four months of infection and in individuals who have suffered repeat attacks of malaria, antimalarial immunoglobulin may be detectable for several years. Infections with species that cause relapsing illness *(P.vivax and P.ovale)* rarely persist longer than three years. Infections with P. falciparum rarely persist longer than one or two years and 99% of patients present within one year of departure from a malarious area.

Globally, malaria presents a significant disease burden with estimates of up to 150 million infections annually and 1-2 million deaths per year, mainly in Sub-Saharan Africa. Although it has not been reported in New Zealand, transfusion transmitted malaria has occurred overseas and on average 3-4 cases are reported each year in the United States. The transmission of malaria by blood transfusion was one of the first recorded incidents of transfusion-transmitted infection and has been reported to occur mainly from red cells, platelets, white cell concentrates, cryoprecipitate and frozen red cells after thawing and washing. Malaria parasites of all species can remain viable in stored blood for at least 1 week and cases of *P.falciparum* malaria have been transmitted by blood stored for 14 days and for 19 days. Of 139 cases of malaria that were reported in New Zealand to ESR (Annual Surveillance Reports) between 2002 and 2004, 20% were *P.falciparum*, 65% were *P.vivax*, 2% *P.ovale*, 2% *P.malariae* and 11% were unclassified.

The Council of Europe Guide to the Preparation, Use and Quality Assurance of Blood Components (CoE Guide) provides two different approaches to the management of donors deemed to be at increased risk of having acquired malaria during overseas travel. Current NZBS policy utilises one of these approaches involving a comprehensive medical and travel history as part of the donor assessment process and the resultant exclusion of cellular blood components from those donors with potential malarial exposure. The period of exclusion varies from 12 months for visitors to malarial areas to three years for those with a documented history of malaria. The second approach to managing "malarial risk donors" involves the identification of these donors together with the results of antibody testing to reduce the period during which donors are excluded from cellular component production.

The number of blood donors whose red cells, fresh plasma and platelets are discarded because of potential malarial risk is increasing as donors travel to malarial risk areas or immigrants from these areas present for donation. By taking the second approach in the CoE Guide to managing "malarial risk donors" NZBS will be able to utilise cellular components from these donors without additional risk and will reduce unnecessary donor and donation loss. NZBS has begun the planning process to introduce Malarial Antibody testing using the Newmarket Laboratories Ltd Malaria EIA test kit. This kitset is currently used by the Australian Red Cross Blood Service and the National Blood Service in England to screen donors who are categorised as malarial risk donors. It is anticipated that implementation of this testing regime will take place during 2007.