

Tuesday 16 October
Masterclasses

0715-0815
Meeting Room 5

HSANZ

Lymphoma Vaccines - Tricks of the Trade

Ronald Levy

Division of Oncology, Stanford University Medical Center, USA

Idiotypic vaccines can be produced by a variety of technologies, including mammalian cells, insect cells, tobacco plants, bacteria, naked DNA and cell-free protein expression. All of these methods result in vaccines that work in preclinical animal models. The results will be compared and contrasted.

Tuesday 16 October
Masterclasses

0715-0815
Meeting Room 6

HSANZ

Tailoring New Myeloma Therapies: How Are They Best Used?

Kenneth C Anderson

Abstract not received at time of going to print

Dr Ken Anderson sponsored by Celgene Pty Ltd

Tuesday 16 October
Masterclasses

0715-0815
Meeting Room 4

ANZSBT **Clinical Aspects of Granulocyte Transfusions**

Jürgen Bux
German Red Cross Blood Service West & University of Bochum, Germany

Despite availability of antibiotics, antifungals and haematopoietic growth factors, infections remain a major threat to neutropenic patients. Although transfusion of granulocytes became more feasible with the development of cell separators, widespread use was hampered by low granulocyte yields, pulmonary transfusion reactions, and conflicting clinical study results. The introduction of the granulocyte colony-stimulating factor (G-CSF) in donor conditioning for granulocyte apheresis increased granulocyte yields. Transfusions of more than 2×10^{11} granulocytes are now routinely possible. In addition, G-CSF prolongs survival of transfused granulocytes by reducing their apoptotic rate, and improves granulocyte antimicrobial function. Gentle cell separation due to progress in apheresis technique and routine leucocyte cross-matching contributed to good tolerance towards granulocyte transfusions. However, required neutrophil dosage, frequency and right moment of granulocyte transfusion in a certain clinical condition have still to be determined in randomized multicentre studies.

Tuesday 16 October
Masterclasses

0715-0815
Meeting Room 8

ANZSBT
Microangiopathies

Thomas Raife
University of Iowa Department of Pathology, Iowa City, IA, USA

In the broadest sense thrombotic thrombocytopenic purpura (TTP) comprises a heterogeneous group of acute microvascular thrombosis syndromes characterized by multiorgan failure and high risk of mortality. There has been much recent progress in understanding the pathogenic underpinnings of several forms of TTP. Elements of endothelial injury and dysregulation of hemostasis are central to the pathophysiology. Plasma exchange is the only well established treatment, although its utility is not understood in some forms of TTP. Recent evidence reveals a critical role of von Willebrand factor (VWF)-mediated platelet thrombosis and deficient activity of the regulatory enzyme ADAMTS13 in the pathogenesis of many cases of TTP. In vitro studies and studies in ADAMTS13 knockout mice have established a pathogenic model of TTP microvascular thrombosis in which stimulated endothelium combined with unregulated VWF-platelet binding result in microvascular thrombosis. TTP associated with ADAMTS13 deficiency is increasingly recognized as a distinct clinical entity. The etiological role of anti-ADAMTS13 autoantibodies as a cause of this form of TTP, and the therapeutic benefit of replacing ADAMTS13, support new approaches to treatment. Immunomodulation with newer agents like rituximab has gained currency in recent years, despite a lack of controlled clinical trials. ADAMTS13 deficiency is an important pathogenic factor, but not the sole cause, of TTP microvascular thrombosis. Current investigations seek to identify additional pathogenic factors, including other enzymes that may regulate VWF.

Tuesday 16 October
Masterclasses

0715-0815
Meeting Room 9

ASTH

Surgery in Haemophilia Complicated by Inhibitors: Options, Decision Process and Limitations

Claude Negrier

Hôpital Edouard Herriot; University of Lyon, France

Patients with severe haemophilia may experience serious orthopaedic complications resulting from repetitive acute bleeding. In patients with inhibitors, surgical procedures have been performed with caution due to the potential difficulty of maintaining haemostasis for an extended period of time to permit wound healing. Surgical or invasive diagnostic procedures represent a challenge to treatment due to the difficulty in controlling bleeding for long periods during and after surgery. Several strategies may be utilised, depending on the inhibitor titre, a previous anamnestic response, the type of surgery, the availability of therapeutic molecules and their specific marketing authorisation. In patients with low-titre inhibitors (<5 BU/mL), haemostasis can be achieved with the replacement of the missing coagulation factor. High doses factor VIII (FVIII)/factor IX (FIX) have to be used to saturate the inhibitor and subsequently increase the plasma concentration of the clotting factor. An anamnestic response may occur after 4-6 days and require a second line therapy with a bypassing agent. Perioperative haemostatic control in haemophilia patients with high-titre inhibitors is often managed with bypassing agents, e.g., Factor Eight Inhibitor Bypassing Activity (FEIBA®; Baxter AG, Vienna, Austria) and recombinant factor VIIa (rFVIIa; Novoseven®, Novo Nordisk, Bagsvaerd, Denmark) since FVIII cannot be given in sufficient quantities to overcome high-titre inhibitors. Both agents have been demonstrated to be effective for perioperative haemostatic cover, although dosing strategies vary greatly. To further improve the knowledge on the use of bypassing agents during surgery, open label, prospective, non-interventional surveillance studies were set up in order to identify best practices in haemostatic management. As none of the current therapies is universally effective, new therapeutic agents, including recombinant porcine FVIII, are under development. In addition, several preclinical and clinical studies are currently carried out for optimizing and individually tailoring the therapeutic regimens using recently developed or revisited coagulation assays.

Tuesday 16 October
HSANZ Symposium: Myeloma
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0830-1000
Arena 1B

0830

Akt as a Potential Therapeutic Target in Multiple Myeloma

Teru Hideshima, Kenneth C Anderson

Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA

Despite conventional therapies with alkylating agents, anthracyclines, corticosteroids, as well as high dose therapy and stem cell transplantation, multiple myeloma (MM) remains incurable due both to intrinsic and acquired drug resistance. Importantly, the BM microenvironment induces growth, survival, and drug resistance in MM cells *via* two different mechanisms: adhesion of MM cells to extracellular matrix proteins confers cell adhesion mediated drug resistance; and cytokines (ie, IL-6, IGF-1, VEGF, SDF-1 α , BAFF) from BM accessory cells trigger MEK/ERK, PI3-K/Akt and/or JAK2/STAT3 signaling cascades. Among these cascades, Akt signaling plays a crucial role in MM cell resistance to conventional therapeutics. Specifically, dexamethasone-induced cytotoxicity is abrogated by Akt activation. Moreover, Akt directly phosphorylates many downstream molecules mediating cell cycle, survival and anti-apoptosis. Besides its direct anti-apoptotic effect, Akt affects two central regulators of cell death, including NF- κ B and p53.

Given the crucial role of PI3K/Akt pathway in MM oncogenesis, Akt and its downstream molecules are rational therapeutic targets for novel therapeutics. Several classes of compounds either directly or indirectly can target Akt. For example, Perifosine, a synthetic alkylphospholipid, inhibits Akt activation. We have shown that Perifosine inhibits baseline and cytokine-induced Akt activity and induces significant cytotoxicity, associated with caspase cleavage in both MM cell lines and patient MM cells resistant to conventional therapeutic agents. Perifosine induces apoptosis even of MM cells adherent to BM stromal cells. Furthermore, it augments dexamethasone, doxorubicin, melphalan, and bortezomib-induced MM cell cytotoxicity. Perifosine also demonstrates significant antitumor activity in a human plasmacytoma mouse model, associated with downregulation of Akt phosphorylation in tumor cells. Most recently, we have shown that Perifosine-induced cytotoxicity is associated with significant downregulation of survivin *via* inhibiting Gsk/ β -catenin pathway. Based upon our preclinical data, a clinical trial of Perifosine with bortezomib is ongoing to improve patient outcome in MM.

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Tuesday 16 October
HSANZ Symposium: Myeloma
Session sponsored by Celgene Pty Ltd

0830-1000
Arena 1B

0905

Novel Therapeutic Strategies in Multiple Myeloma

Kenneth C Anderson
Dana-Farber Cancer Institute, Boston, MA USA

Thalidomide, lenalidomide, and Bortezomib are three agents targeting the tumor cell in its bone marrow microenvironment in both laboratory and animal models, which have rapidly translated from the bench to the bedside and FDA approval. Each achieved responses in relapsed refractory MM, and then increased extent and frequency of response, as well as prolonged progression free and overall survival, when used as initial therapy. Thalidomide has prolonged overall and progression free survival, and both lenalidomide and bortezomib are under evaluation, as maintenance therapies. Clinical trials of novel single agents in phase I/II clinical trials in MM include: HuLuc63 monoclonal antibody targeting CS-1, TKI 258 targeting FGFR3, SF1126 targeting Akt; proteasome inhibitors NPI0052 and Carfilzomib; and histone deacetylase inhibitor LBH589. Preclinical studies have defined combinations to enhance cytotoxicity, overcome drug resistance, and reduce side effects. Bortezomib has been combined with doxil (FDA approved), heat shock protein 90 inhibitor (phase III), as well as with lenalidomide, Akt inhibitor perifosine, LBH589, mTOR inhibitor CCI 779, and farnesyltransferase inhibitor Zarnestra (phase I/II). Lenalidomide has been combined with dexamethasone (FDA approved), as well as with proteasome inhibitors and humanized monoclonal antibodies (phase I/II). High throughput assays can assess the additive or synergistic cytotoxicity of combination therapies against MM cells, both alone and in the BM microenvironment. Correlative science will define those patients most likely to respond, determine mechanisms of sensitivity versus resistance, and further inform combination protocols. Already three (lenalidomide, dexamethasone, and perifosine) and four (bortezomib, dexamethasone, doxil, and lenalidomide) drug phase I/II clinical trials are underway. This new paradigm of scientifically-based combination therapy targeting the MM cell in its microenvironment has already increased response rate and extent, as well as progression free and overall survival; and, as in acute lymphocytic leukemia, Hodgkins disease, and testicular cancer, has curative potential in MM as well.

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Tuesday 16 October
HSANZ Symposium: Myeloma
Session sponsored by Celgene Pty Ltd

0830-1000
Arena 1B

0925

The Role of Stem Cell Transplantation in Multiple Myeloma in 2007

Jean-Luc Harousseau

Department of Hematology, University Hospital Hotel-Dieu, Nantes, France

1) Until now Autologous Stem Cell Transplantation (ASCT) is considered the standard of care for newly diagnosed patients with Multiple Myeloma (MM) up to 65 years of age. This is mostly due to the superiority of High-Dose Therapy compared to conventional chemotherapy in terms of complete remission (CR) rate, progression-free survival (PFS) and overall survival in the IFM 90 and the MRC 7 randomized trials. However recently developed combinations with novel agents (Thalidomide, Bortezomib, Lenalidomide) yield CR rates and PFS that are comparable to those achieved with ASCT. Since ASCT can also be a useful salvage treatment for relapsed MM, some investigators are currently discussing the interest of frontline ASCT. On the other hand the overall results of ASCT have already been improved in the past 10 years by double ASCT and by the introduction of novel agents. Novel agents can be used in 3 different indications:

- Prior to ASCT (induction treatment) Combinations with Thalidomide increase the response rate compared to dexamethasone alone or to VAD. Combinations with Bortezomib increase not only the response rate but also the CR rate both prior and after ASCT and could therefore prolong PFS as well.
- In the preparative regimen in combination with High-Dose Melphalan
- As maintenance therapy after ASCT. Results of the IFM 99.02 study show the benefit of maintenance therapy with Thalidomide after ASCT and have been confirmed by the Australian randomized studies.

2) In the context of allogeneic SCT myeloablative conditioning regimens have been almost abandoned due to excessive toxicity. Results of tandem ASCT/reduced intensity conditioning allogeneic SCT are encouraging but 1 year transplant-related mortality is still 10-15% and the balance risk/benefit of chronic GVHD remains a problem.

Dr Jean-Luc Harousseau sponsored by Pharmion

Tuesday 16 October
ANZSBT Free Communications I
Sponsored by Haemonetics

0830-1000
Central Room

O59 **RhD Genotyping**

0830

Mary Zmijarevic
Royal Melbourne Hospital, Victoria, Australia

Aim

To establish a method to determine the RhD genotype.

Method

Sixty three blood samples were RhD typed with commercial anti-D. DNA was then extracted from white cells. Within the RhD gene, exons 4, 7 and 10 amplified by PCR and analysed by agarose gel electrophoresis. Samples were also tested for the RhD (W16X) on exon 1, RhD Ψ on exon 4 and RhD-CE (8,9)-D mutation. Real-time PCR was also performed on the samples. Regions within exon 4 (RhD Ψ), 5 and 10 were amplified.

Results

The RhD groups were tested by serological methods. Out of a total of 63 samples, 32 samples were RhD positive and 31 were RhD negative. Samples were analysed by agarose gel electrophoresis and real time PCR. Results were consistent with their phenotype. No mutations were detected.

Conclusion

The RhD gene is about 60 kilobases in length and consists of 10 exons and introns. Typically, the RhD negative phenotype results from complete deletion of the RhD gene. Variant haplotypes may deviate from all, part of the RhD gene present or mutations within the gene.

While no discrepancies or mutations were detected in this study, one of the most common variants seen in people of African origin is the RhD Ψ . Approximately 66% of RhD negative Black Africans have this mutation. They serologically type as RhD negative, but contain all 10 exons of the RhD gene. The gene is inactive due to a 37 bp duplication in exon 4, a nonsense mutation in exon 6 and a single nucleotide polymorphism in exons 4 and 5.

Due to the ethnic diversity in the Australian population, capturing mutations is critical to the correct diagnosis. When selecting segments within the RhD gene for amplification, it is essential to choose regions that also capture mutations common to the population analysed.

Tuesday 16 October
ANZSBT Free Communications I
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0830-1000
Central Room

O60 0845

A Comparison of the Oxidative Responses of Ovine and Human Neutrophils

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¹ Australian Red Cross Blood Service, Brisbane, Australia

² Critical Care Research Group, The Prince Charles Hospital, Brisbane, Australia

³ The Prince Charles Hospital, Brisbane, Australia

⁴ Princess Alexandra Hospital and Queensland University of Technology- Institute of Health and Biomedical Innovation, Brisbane, Australia

⁵ University of Queensland, Brisbane, Australia

Aim

Neutrophils are one of the key effector cells in the development of Transfusion Related Acute Lung Injury (TRALI), a potentially fatal complication in blood transfusion. As a prelude to the development of an ovine model of TRALI, our aim was to compare the function of ovine neutrophils to human neutrophils.

Methods

Neutrophils were isolated from fresh human citrated blood (n=96) and from fresh ovine citrated blood (n=10). Isolated neutrophils were immediately tested on the superoxide anion release assay, and the maximum rate of O_2^- released was measured. Human neutrophils were incubated with combinations of buffer, Platelet Activating Factor (PAF), and N-formylmethionyl-leucyl-phenylalanine (fMLF). Ovine neutrophils were incubated with combinations of buffer, PAF, fMLF, and Phorbol 12-Myristate 13-Acetate (PMA). Results were analysed with an unpaired two-tailed t-test, with a confidence limit of 99%.

Results

| | Nil | PAF | fMLF | PAF + fMLF | PMA | PAF + PMA | PMA + PAF | PAF & PMA |
|-------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|
| Human | 0.189 (±0.25) n=96 | 0.23 (±0.23) n=94 | 1.96 (±1.43) n=92 | 8.20 (±2.09) n=91 | N/A | N/A | N/A | N/A |
| Ovine | 0.25 (±0.08) n=10 | 0.67 (±0.29) n=10 | 0.54 (±0.19) n=9 | 1.53 (±1.25) n=9 | 1.65 (±0.63) n=10 | 1.89 (±1.09) n=10 | 2.10 (±0.64) n=10 | 4.52 (±1.46) n=5 |
| P-Value | 0.4442 | <0.0001 | 0.0023 | <0.0001 | N/A | N/A | N/A | N/A |
| Significant | No | Yes | Yes | Yes | N/A | N/A | N/A | N/A |

The average maximum rates of superoxide anion release (\pm SD) from ovine and human neutrophils in response to various stimulants. Units are $nmol O_2^- / 2 \times 10^5$ neutrophils / min. Human neutrophils responded both to fMLF alone and to fMLF following PAF, while ovine neutrophils did not ($P < 0.01$). PAF added simultaneously with PMA significantly increased the rate of O_2^- production ($P = 0.0001$), but this was still significantly lower than the response of human neutrophils to fMLF following PAF ($P = 0.0002$).

Conclusions

The response of ovine neutrophils to PAF and fMLF, both well know activators of human neutrophils, is significantly lower. Human neutrophils demonstrated a two-event oxidative response where PAF initially primed neutrophils for enhanced secondary fMLP-induced activation. Our data indicated that ovine neutrophils did not exhibit a similar two-event response, and thus sheep may have limited use as an animal model for TRALI.

Tuesday 16 October
ANZSBT Free Communications I
Sponsored by Haemonetics

0830-1000
Central Room

O61

0900

Rh D Immunoglobulin Prophylaxis. A Comparison of Two Tertiary Women's Hospitals

Helen Savoia¹, Selina Northover¹, Trudi Verrall²

¹ *Royal Children's Hospital, Melbourne, VIC, Australia*

² *Children, Youth and Women's Health Service, Adelaide, SA, Australia*

Background

Rh D immunoglobulin (anti-D) is used to protect Rh D negative women against the development of Rh D antibodies which may cause Haemolytic Disease of the Newborn in subsequent pregnancies. In Australia, the National Health and Medical Research Council (1999) evidence based guidelines have been implemented using a staged approach. The final stage of the program was implemented in March 2006, leading to anti-D being administered for (1) potentially sensitizing antenatal events, (2) antenatal prophylaxis and (3) post partum prophylaxis.

Aim

The aims of the audit were to determine:

1. If the hospitals were compliant with the National Blood Authority Guidelines with regards to the administration of anti-D for antenatal and post partum prophylaxis.
2. Whether the hospitals had robust documentation of anti-D administration that would allow for traceability and clear evidence that anti-D had been administered.

Method

The audit was conducted by reviewing the medical record of all Rh D negative women delivering during a one month period during 2006. 57 Rh D negative women delivered at hospital A during the audit month. 6 were excluded for further analysis because of pre-existing Rh D isoimmunisation. 52 Rh D negative women delivered at hospital B during the audit month. 1 was excluded due to pre-existing RH D isoimmunisation. This resulted in 51 women being eligible for analysis from each site.

Results

This audit demonstrates that overall compliance with the guidelines for administration of anti D in obstetrics is well adhered to. Compliance is best for postnatal administration with both institutions approaching 100%. Compliance is poorest at 34 weeks gestation (82 and 80% respectively) and reasons for this need further exploration. A small number of women are refusing anti-D administration and reasons for this refusal need further exploration.

Documentation of anti-D administration was greatest for the date of administration (>95%), dosage was documented approximately 80% of the time, signature was poor at 28 and 34 weeks at hospital B (60-80%). However batch numbers were the most poorly documented ranging from 20-80%. Documentation requires improvement particularly in the area of Batch number recording as this is vital for product traceability.

Postnatal testing of cord blood of infants from Rh D negative women was well complied with. It is of interest to note that Hospital B did not routinely perform any estimation of fetomaternal haemorrhage.

Tuesday 16 October
ANZSBT Free Communications I
Sponsored by Haemonetics

0830-1000
Central Room

O62

0915

Risk Factors Associated with the Development of Microvascular Bleeding in Association with Massive Transfusion

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¹ *Transfusion Service, Flinders Medical Centre, Bedford Park, South Australia, Australia*

² *Intensive & Critical Care Unit, Flinders Medical Centre, Bedford Park, South Australia, Australia*

Background and Aim

Microvascular bleeding may occur in patients who require massive blood transfusion in the settings of emergency surgery and trauma. It is a serious and often terminal event unless the underlying coagulopathy is corrected. Presently there are no definitive tests to predict which patients will develop microvascular bleeding. The aim of this study was to identify risk factors for microvascular bleeding and mortality associated with massive transfusion.

Materials and Methods

Patients who had received more than 10 units of blood in a 24 hour period during 1995-1996 or 2005 were included in the study. Case records of 40 patients from 1995-1996 and 18 from 2005 were reviewed. The association between demographic details, laboratory data including haemoglobin, platelet count, International Normalised Ratio (INR), thromboplastin time (aPTT ratio [test/control]), fibrinogen and blood product usage, and microvascular bleeding and mortality was examined using separate logistic regression analyses.

Results

The overall incidence of microvascular bleeding was 27.5% (n=16) with the little variance between 1995-1996 (28.2% [n=11]) and 2005 (31.3% [n=5]). Using univariate analysis the risk factors associated with microvascular bleeding included pH<7.1 (p=0.003), INR≥2, (p=0.009) and thromboplastin time (aPTT ratio ≥2.2, p=0.009). Patient survival in 2005 was 80% as compared to 18% in 1995-1996 (p=0.01).

The overall survival rate was 70.7%. Univariate analysis indicated the risk factors associated with mortality included pH <7.1 (p=0.001), INR≥2 (p=0.003) and thromboplastin time (aPTT ratio ≥2.5, p=0.001).

Conclusion

Microvascular bleeding is associated with acidosis, dilution and consumption of coagulation factors. Close laboratory monitoring, early recognition of microvascular bleeding, correction of acidosis and aggressive supportive care with fresh frozen plasma, platelets and cryoprecipitate may improve patient outcomes.

Tuesday 16 October
ANZSBT Free Communications I
Sponsored by Haemonetics

0830-1000
Central Room A

O63

0930

Recent Trends in the Incidence of Acute HIV, HCV and HBV Infection in Repeat Blood Donors

CR Seed, A Cheng, P Kiely, AJ Keller
Australian Red Cross Blood Service

Aim

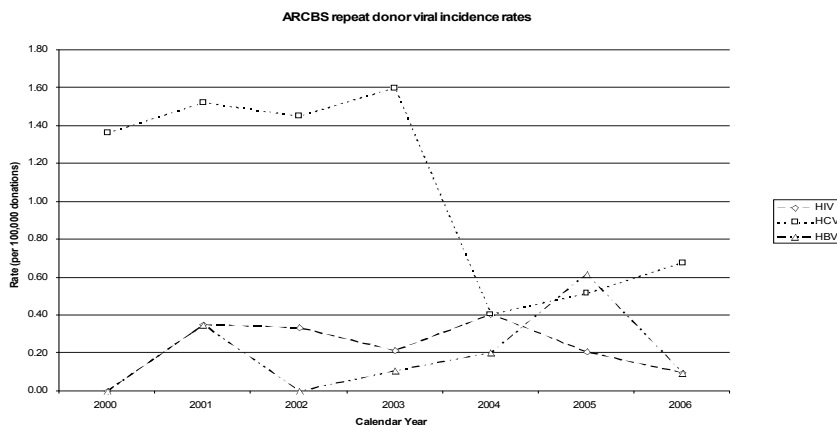
The risk of transfusion transmitted HIV, HCV and HBV infection in Australia is very low due to sensitive testing methods and effective behavioral based donor exclusion criteria. The residual risk is almost exclusively due to donors with recent (incident) infection because they are much more likely to be in the window period (WP) of infection i.e. before they can be detected by the screening test applied. In order to investigate recent incidence rate trends in the ARCBS donor population we analysed national screening data for HIV, HCV and HBV in repeat blood donors since 2000.

Methods

The incidence rate by calendar year was derived based on the number of repeat blood donors attending between 01 January 2000 and December 31 2006 who 'seroconverted' i.e. were confirmed positive on the current donation but had previously been negative for the same virus. Screening tests applied during the period were as follows; HIV-1/2 antibody and RNA (from June 2000), HCV antibody and RNA (from June 2000) and HBsAg. Incidence was expressed as rate per 100,000 repeat donations.

Results

The incidence rates for HIV and HBV have been relatively stable since 2000 without any discernible trend. In contrast the incidence rate for HCV was stable from 2000 to 2003 but declined significantly ($p=0.007$) by almost 3 fold in 2004 remaining relatively stable since.



Conclusion

The low and relatively stable incidence rate of HIV and HBV over the period is encouraging and suggests that repeat blood donors understand and usually avoid risk behavior for these viruses that might lead to infection. The significant decline in 2003/2004 for HCV (predominantly in NSW and Qld.) to a rate of comparable magnitude to HIV and HBV signals a potential shift in donor behavior perhaps directly related to the significant public awareness campaign associated with HCV.

Tuesday 16 October
ANZSBT Free Communications I

Sponsored by Haemonetics

0830-1000
Central Room

O64
Massive Blood Loss Management - Experience in a Tertiary Hospital
Session Sponsored by Haemonetics

0945

Lakshmi Nath,^{1,2} Chris Hogan,¹ Erica Wood,^{1,2} Michael Haeusler¹

¹*Diagnostic Haematology, Royal Melbourne Hospital, Melbourne*

²*Transfusion Medicine Services, Australian Red Cross Blood Service, Melbourne*

Aim

Few Australian data on massive blood transfusion (MBT) and patient outcomes have been published. We analysed data for MBT episodes in the first 24 hours after presentation to the Emergency Department at The Royal Melbourne Hospital, to identify factors contributing to haemostatic complications and outcomes, and to suggest areas for focused management improvement.

Methods

Review of MBT episode data for 100 patients receiving ≥ 10 red cell units in the first 24 hours from 1/1/04 to 31/3/07 and for whom full documentation was available. Data were extracted from our pathology information system (Kestral) and analysed for clinical and statistical (student's t-test) significance.

Results

100 MBT patients were identified. Overall survival rate was 71%, measured at 48 hours after admission. The coagulopathy rate was 60 % in patients receiving ≥ 10 red cell units and 82% when receiving >30 units, emphasising the risk of coagulopathy in MBT. This was associated with acidosis and increased mortality. There was significant impact on our hospital inventory, with usage of 1992 red cell units, 308 platelet doses, 721 units of fresh frozen plasma, and 1782 cryoprecipitate units, in correcting bleeding/haemostasis in this group, emphasising the need to maintain sufficient inventory for management of MBT.

Conclusion

The combination of transfusion support to improve haemostasis, effective rewarming procedures, improved application of damage control surgical measures, better communication and a proactive advisory role by the transfusion medicine team at the hospital and the Australian Red Cross Blood Service contributes to improving the management of patients requiring MBT. These data can be used for clinical supply planning, for reviewing clinical effectiveness of product use, and to some extent, also act as a measure of the quality of this significant section of our health care delivery.

Tuesday 16 October
ANZSBT Free Communications 2

0830-1000
Meeting Room 7

O65

0830

Troublesome Stowaways: Severe Haemolysis Resulting from Passenger Lymphocyte Syndrome in all Three Recipients of Organs from a Donor with Multiple Red Cell Alloantibodies

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³*Transfusion Service, Flinders Medical Centre, Adelaide*

⁴*Respiratory Transplant Unit; Alfred Hospital, Melbourne*

Background

Severe haemolysis secondary to solid organ transplantation is rare. We present three recipients of transplanted organs derived from a donor with multiple red cell (RC) alloantibodies. All developed significant haemolysis.

Case series

Donor: A 54 year old male, blood group O negative (rr) with a positive antibody screen due to anti-D and anti-k.

1: Right lung recipient, a 61 year old male with emphysema (blood group O positive [R2r]). From post transplant day (PTD) 0 to 10 his haemoglobin (Hb) fell from 126g/L to 69g/L without bleeding and with elevated haemolytic markers. A positive direct antiglobulin test (DAT) due to IgG was noted and anti-D eluted. Management included increased immunosuppression and transfusion of two RC units (rr, k positive), with post transfusion Hb 96g/L. He subsequently stabilised.

2: Left lung recipient, a 59 year old female with emphysema (blood group O positive [R1r]). From PTD 0 to 10, her Hb fell from 91g/L to 73g/L with elevated haemolytic markers. The antibody screen and elution demonstrated anti-D. She was transfused with two RC units (rr, k positive), with a post transfusion Hb of 97g/L, and stabilised.

3: Liver recipient, a 45 year old male with Hepatitis B (blood group B positive [R1R1]). From PTD 0 to 13 his Hb fell from 102g/L to 64g/L without bleeding. The DAT was positive (IgG) at day 4. At PTD 16 numerous spherocytes, marked polychromasia and occasional nucleated RCs were present. Anti-D+B+?C were detected in the plasma and anti-C+D+k+B were found in eluates. He was managed with increased immunosuppression and transfusion of two O negative (rr, k negative) RCs. Post transfusion Hb was 94g/L.

Conclusion

A positive donor RC antibody screen should prompt close monitoring of solid organ recipients for development of alloimmune haemolysis. Alloantibodies against high incidence antigens (anti-k in this case) further complicate transfusion support.

Tuesday 16 October
ANZSBT Free Communications 2

0830-1000
Meeting Room 7

O66 **Errors of Patient Identification Are Common During the Collection and Administration of Fresh Blood Components**

0845

Simon Brown¹, Sandra Blake², John Wakefield², Tony Ghent^{3,4}, Gina Clare¹, Margot Ovenden¹, John Rowell³, Sue Williams³

¹Queensland Blood Management Program, ²Queensland Health Patient Safety Centre, and ³Queensland Health Pathology Service; Royal Brisbane and Women's Hospital, Brisbane, Queensland and ⁴Gold Coast Hospital, Southport, Queensland

Aim

An audit was undertaken in four Queensland hospitals, which receive approximately 25% of red cells issued in Queensland, to assess clinical practice against the National Guidelines for the administration of blood and blood components.

Method

An audit tool was designed based on the procedures and steps outlined in the national guidelines for the collection and administration of blood and blood products (ANZSBT/RCNA 2004). Audit teams visited 4 hospitals and observed staff collecting and administering blood components.

Result

In total 39 audits were completed during eight visits. Errors leading to patient misidentification due to poor adherence to the guidelines were common at both the point of collection and administration of blood components. In 21% of audits no written patient identification (PID) was taken to the collection point, resulting in 28% of audits identifying no check of PID against the component collected. At the point of administration 27% of patients had no wristband (WB), and 34% avoidable errors of verbal confirmation of PID occurred. As a result, the verbal PID was checked against the WB in only 37% of audits. 11% of components were not spiked at the bedside, and a many 'bedside' checks were performed away from the patient. However, there was excellent adherence to other bedside checks and for the number of staff performing the checks. During 1 audit the staff identified mislabelling of two blood components by carrying out the correct checks at the time of collection.

Conclusion

Errors of patient identification are common during the administration of blood components and result in the incorrect blood component being transfused (IBCT). Haemovigilance systems have confirmed IBCT as the most frequent incident relating to transfusion practice. Ensuring staff correctly use verbal verification of identification checked against the wristband would contribute to a significant reduction in non-harm incidents and improve patient safety.

Tuesday 16 October
ANZSBT Free Communications 2

0830-1000
Meeting Room 7

O67

0900

NZBS Sample and Request Form Labelling Errors Database – One Year On

Simon Benson

New Zealand Blood Service, Auckland, New Zealand

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 six blood banks for recording labelling errors associated with pretransfusion testing requests. The standardised list of errors to be reported is based on NZBS sample and request form labelling requirements and ANZBST *Guidelines For Pretransfusion Laboratory Practice*. Errors are recorded on a standard form and data subsequently entered into a Microsoft Access™ database for analysis and reporting.

During the period 1May 2006 to 30 April 2007 146,195 requests for pretransfusion testing were received, of which 6,125 (4.2%) were reported to have one or more errors in sample and/or request form labelling. The number of errors per request ranged from one to five, with the clear majority (88.8%) having a single error.

Of the 6,907 errors reported 79.9% were due to the five most prevalent errors which are, patient details - discrepancy between sample and form (20.0%), sample not signed (17.3%), sticky label on sample (15.6%), missing/incomplete details (14.4%), and declaration not signed (12.6%).

Three main indicators have been chosen for wider reporting, namely error rate per 1000 requests, sample recollect rate and incidence of 'wrong blood in tube' events. The aim is that data is available for discussion at each the associated hospitals, for example at the hospital transfusion committee.

The 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 NZBS blood banks. This knowledge provides a unique opportunity for raising the awareness of what errors are occurring and where, awareness which will hopefully precipitate a reduction in numbers.

Tuesday 16 October
ANZSBT Free Communications 2

0830-1000
Meeting Room 7

O68 **Antibody Titration Methods in ABO Incompatible Renal Transplantation**

0915

Helen Fairweather¹, Michael Haeusler¹, Solomon Cohny², Chris Hogan¹

¹*Department of Diagnostic Haematology, Royal Melbourne Hospital, Victoria*

²*Department of Nephrology, Royal Melbourne Hospital, Victoria*

Aim

The Royal Melbourne Hospital has undertaken Australia's first ABO incompatible renal transplantation program. This provides challenges for the laboratory providing interpretive data to aid clinical decision making. The paucity of literature on laboratory methods, in combination with variations in laboratory techniques, testing platforms and titre definitions makes data comparison difficult. We outline the experience of our laboratory.

Methods

Between September of 2005 and July of 2007 16 ABO incompatible renal transplants were performed. Anti-A and anti-B antibody titres were performed on all patients using three different platforms (manual test tube and 2 microcolumn techniques: DiaMed and Ortho BioVue) and three different phases (Indirect antiglobulin testing (IAT) and saline testing at room temperature and 37 °C). Comparison was made between platforms and phases of testing during transplantation and in response to interventions.

Results

Pre-transplantation titres were performed against both donor and reference cells. In only 3 patients titres were not comparable, of which 2 donors had A subgroups. Among test tube methods the IAT proved the most sensitive method. All phases reacted similarly to interventions. For IAT titration test tube methods were more sensitive than the microcolumn techniques. Of the microcolumn techniques Ortho BioVue was more sensitive than DiaMed. All 3 IAT methods responded similarly during conditioning and post transplantation. All titres, irrespective of method, fell more in response to column immunoadsorption than plasma exchange. Only one patient experienced antibody mediated rejection, likely to be secondary to a non-ABO antibody. All patients are alive with functioning grafts.

Conclusion

Testing against reference cells is acceptable for patients in whom pre-transplant titres with donor and reference cells are comparable. The IAT is the most sensitive test tube phase of titre determination and is a suitable surrogate saline methods. Test tubes are more sensitive than microcolumn techniques for titration using IAT, although all platforms respond to interventions similarly making them all potentially suitable methods

Tuesday 16 October
ANZSBT Free Communications 2

0830-1000
Meeting Room 7

O69

0930

Investigation of the Epitope Specificity of Antibodies to Variant MNS Blood Group Antigens in South Asia

Robert Flower

Pacific Laboratory Medicine Services, RNSH and University of Sydney, Sydney, Australia

Introduction

Several phenotypes in the MNS system, often generically termed anti-Mi(a), have a significant prevalence in Asian populations. Antibodies against these antigens cause transfusion reactions and haemolytic disease of the newborn. It is difficult to determine the clinical importance or epitope specificity as most of these antibodies to variant MNS cell phenotypes have mixed specificities. Use of peptide-inhibition to define vMNS antibodies was reported in 1991 and analysis by ELISA in 2000.

Materials and Methods

Serums with anti-Mi(a) activity from five different Asian countries were diluted in PBS and tested by peptide-ELISA with peptides mimeotopes representing vMNS/Mi(a) antigens: 14 peptides were tested in both N-biotinylated & C-biotinylated forms (Mimotopes, Australia). Peptides were bound to neutravidin on 96-well plates and antibody-binding quantified by protein G-peroxidase activity. A positive result was a mean absorbance for the test wells twice the absorbance value of controls.

Results

Fourty percent of sera found to be Mi(a) pos by IAT were not positive by ELISA. Fourteen percent of serums showed single reactivity with the Mut peptides, 10% with the Mur peptides only, 5% with the Tsen or Hil peptides and 2% with a GpA24-34 peptide. Nine percent reacted with both Mut and Mur and 20% of serums reacted with other multiple peptide combinations.

Conclusion

An overview of the epitope reactivity in anti-Mi(a) serums was provided by the results for the serums that were Mi(a) positive by both IAT and peptide ELISA. The majority of the antibodies detected by peptide ELISA were against GP variants resulting from B-A-B gene conversions found in the Asian population, GP.Mur and GP.Bun; some of these epitopes are also found on less common variants also found in Asia GP.Hut and GP.Hil. Peptide mimeotopes can be used to represent variant MNS antigens and may provide a basis for classifying their clinical importance.

Tuesday 16 October
ANZSBT Free Communications 2

0830-1000
Meeting Room 7

O70

0945

A Potential New Technique to Study Epitope Specificity of Antibodies to Variant MNS Blood Group Antigens

Steve Henry¹, Robert Flower²

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² *Pacific Laboratory Medicine Services, RNSH and University of Sydney, Sydney, Australia*

Introduction

Obtaining cells with Miltenberger blood group variations is problematic in the construction of antibody screening and identification panels. Using KODE™ technology we modified a series of cells to express a range of Miltenberger peptides.

Materials and Methods

Serums with anti-Miltenberger activity from five different Asian countries were tested against a panel of KODE™ red cells expressing Miltenberger peptides.

Results

Preliminary results established the ability of KODE™ technology to express serologically active Miltenberger peptides on antigen negative red cells. Extended serological results including sensitivity and specificity obtained from a variety of peptides expressed on a several different KODE™ backbones will be shown.

Conclusion

Miltenberger peptides as KODE™ constructs expressed on the surface of Miltenberger negative cells have the potential to be a basis for classifying anti-Miltenberger antibodies.

Tuesday 16 October
ASTH Symposium: Platelets

0830-1000
Central Room C

Inherited Platelet Disorders

Alan T Nurden and Paquita Nurden

Centre de Référence des Pathologies Plaquettaires, Plateforme Technologique et d'Innovation Biomédicale, Hôpital Xavier Arnozan, Pessac, France

Inherited disorders of platelets constitute a group of rare diseases giving rise to bleeding syndromes of varying severity. Studies on platelet function defects have yielded much information on how a hemostatically active thrombus forms and have identified targets for anti-thrombotic therapy. The classic disorders of platelet adhesion and platelet aggregation are Bernard-Soulier syndrome (BSS) (with giant platelets) and Glanzmann thrombasthenia (GT). In BSS, mutations in the genes encoding glycoprotein (GP)Ib α , GPIb β or GPIX lead to a block in GPIb-IX-V maturation and a molecular deficiency of this essential adhesion receptor for von Willebrand factor (VWF). Rare mutations in a disulfide loop in GPIb α result in up-regulated binding of VWF and platelet-type von Willebrand disease. In GT, a wide panoply of mutations in the genes encoding the α Ib β 3 integrin block its biosynthesis and surface expression while up-regulating mutations in the disulfide-rich EGF domains of β 3 allow spontaneous binding of fibrinogen. Among inherited disorders of secretion, the gray platelet syndrome (GPS) results from defective α -granule maturation and the inability to store growth factors and cytokines. Heterogeneity in GPS extends to a subgroup with spontaneous metalloprotease-mediated cleavage of GPVI and a more severe platelet aggregation defect. In the Hermansky-Pudlak and Chediak-Higashi syndromes, defects in pathways of dense granule (and melanosome) biogenesis lead to a deficient platelet storage pool of nucleotides and serotonin. Despite advances in the understanding of how signalling pathways combine to induce the platelet aggregation and secretory responses, only for P2Y₁₂ (ADP) and TP α (thromboxane A₂) receptors has a clear phenotype been linked to gene defects. Yet, reductions in the platelet aggregation response to selected agonists may be common in trauma-linked bleeding. The key question is how to identify the molecular lesions in patients with abnormal thrombus stability. Specific haplotypes resulting in a greater bleeding risk need to be identified.

Tuesday 16 October
ASTH Symposium: Platelets

0830-1000
Central Room C

0945

Acquired Platelet Disorders

Chris Ward

Department of Haematology, Royal North Shore Hospital, Sydney, NSW, Australia

Platelet function disorders present with unexpected, even life-threatening bleeding, despite normal platelet counts. Bleeding is predominantly mucosal, or at the site of injuries, but deep muscular or intracranial haemorrhages can occur. Acquired platelet function disorders are much more common than inherited defects. Common causes include uraemia, and hepatic disorders, where hypersplenism and coagulopathies increase the risk of bleeding. Haematological conditions such as myeloma and other monoclonal gammopathies can affect platelet function. The platelet defects associated with myelodysplastic syndromes and myeloproliferative disorders can be particularly problematic. Drugs are the commonest cause of acquired platelet function defect – these include the widely used antiplatelet agents, aspirin and clopidogrel, as well as antibiotics, diuretics, alcohol and a range of psychiatric drugs. An approach to the diagnosis and management of acquired platelet function defects will be presented. If supplementary therapies, such as desmopressin and tranexamic acid, are not effective or contraindicated, then platelet transfusion may be required.

Tuesday 16 October

1030-1130

HSANZ Free Communications 5: Haematopoietic Stem Cell Transplantation I

Session sponsored by Schering-Plough Pty Ltd

Arena 1B

O71

1030

CMV Reactivation and KIR Haplotype Following Sibling Allogeneic Haemopoietic Stem Cell Transplantation

Susan Heatley^{1,4}, Charles Mullighan^{2,3}, Kathleen Doherty¹, Silke Danner¹, Uwe Hahn³, George Car⁴, Ji-Yao Sun⁵, David Senitzer⁵, Kenneth Bradstock⁶, Anthony Schwarzer⁷, Jeff Szer⁸ and Peter Bardy^{1,3}

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Aim

Cytomegalovirus (CMV) reactivation is an important cause of morbidity following allogeneic haematopoietic stem cell transplantation (HSCT). Natural killer (NK) cells are important in innate immunity including antiviral responses. NK cells express an array of activating and inhibitory receptors known as Killer cell Immunoglobulin-like Receptors (KIR). KIR haplotype nomenclature defines the A haplotype as carrying 2DL1, 2DL3, 3DL1, 2DS4 genes, with all other combinations denoted the B haplotype. While the B haplotype, containing more activating KIR, has been associated with reduced CMV reactivation post HSCT, other studies have been contradictory. The aim of this study was to clarify the role of donor KIR in CMV reactivation.

Method

Associations between KIR haplotype and CMV reactivation were examined in a cohort of 147 HLA-matched sibling HSCT. CMV IgG-positive recipients (n=99) were monitored by PCR for reactivation and managed by either pre-emptive (n=54) or prophylactic (n=45) antiviral regimens. KIR genotyping was performed by multiplex PCR-SSP.

Results

Of IgG-positive recipients, 45% reactivated CMV, and those on the pre-emptive strategy were more likely to reactivate CMV than those treated with suppressive regimens (55% v 33%, p = 0.027). The frequencies of the KIR A and B haplotypes in the entire cohort were 0.257 and 0.743 respectively. Recipients whose donors carried the A haplotype were significantly more likely to reactivate CMV than those whose donors possessed the B haplotype (65% v 40%, p = 0.049). However when this was further stratified, in patients receiving pre-emptive treatment, there were no significant differences according to donor KIR haplotype (63% v 53%). In contrast, CMV reactivation in patients receiving prophylactic antivirals increased when donors carried the A rather than B haplotype (66% v 25%, p = 0.018).

Conclusion

These findings suggest that donor activating KIR reduced the risk of CMV reactivation in IgG-positive recipients receiving suppressive CMV prophylaxis.

Tuesday 16 October

1030-1130

HSANZ Free Communications 5: Haematopoietic Stem Cell Transplantation I

Session sponsored by Schering-Plough Pty Ltd

Arena 1B

O72

1045

The Contribution of Bone Marrow Derived Cells to Solid Organ Malignancies After Allogeneic Stem Cell Transplantation

Daniel Worthley^{1,2}, Peter Bardy^{3,4}, Peter Browett⁵, Simon Durrant⁶, Sarah Moore³, Charles Mullighan⁷, Ian Nivison-Smith⁸, Andrew Ruszkiewicz³, Robyn Western⁶, Graeme Young², Michael Michael².

¹Conjoint Gastroenterology Laboratory QIMR QLD, ²Flinders Medical Centre SA, ³IMVS SA, ⁴Royal Adelaide Hospital and The Queen Elizabeth Hospital SA, ⁵University of Auckland New Zealand, ⁶Department of Oncology and Haematology RBWH QLD, ⁷St. Jude Children's Hospital US ⁸Australian Bone Marrow Transplant Registry NSW

Aim

Perform fluorescent *in-situ* hybridization (FISH) to determine if male (Y-chromosome containing) bone marrow-derived donor cells (BMDs) contribute to solid organ cancers in female recipients following allogeneic-stem cell transplantation (allo-SCT). Our study is founded on recent animal research as well as two human case-reports suggesting BMDs directly contribute to solid organ carcinogenesis.

Methods

We interrogated the 3,943 recorded allo-SCTs from the Australasian Bone Marrow Transplant Recipient Registry, to identify 127 cases of secondary malignancy, including skin cancers. Eighteen of these satisfied our criteria; female recipients of male grafts. The paraffin blocks for 16 of these secondary cancers were available for H&E light microscopy as well as FISH analysis for the X and Y chromosomes. Any Y-chromosome within malignant cells was further evaluated to confirm cell type. One case remains to be processed, but results will be available by the time of HAA 2007. Details of the transplant, previous male pregnancies, and nature of the secondary cancer were recorded.

Results

In our study the secondary cancers included basal and squamous cell skin carcinomas, cervical intraepithelial neoplasia, melanoma and gastric cancer. The mean interval between allo-SCT and diagnosis of secondary cancer was 3.1 years. In the 15 tumour samples analysed, Y-chromosomes were found in all nucleated haematopoietic cells, with a dense inflammatory infiltrate invading several of the tumours. No Y-chromosomes, however, have yet been identified in any of the malignant cells. In all of the cancer cells only the recipients' sex chromosomes were evident. The gastric cancer case, which is potentially the most valuable, remains to be analysed.

Conclusions

From this series, the largest of its type, BMDs did not contribute directly to solid organ cancer following allo-SCT. This does not exclude their contribution to cancer in other settings.

Tuesday 16 October

1030-1130

HSANZ Free Communications 5: Haematopoietic Stem Cell Transplantation I
Session sponsored by Schering-Plough Pty Ltd

Arena 1B

O73

1100

Delayed Onset GVHD in Reduced Intensity Conditioned Haematopoietic Stem Cell Transplant Recipients is Associated with Persistence of Host Cells

Brie E Turner¹, Melinda E Kambouris¹, Janusz Lange¹, Ann M Burns¹, Derek NJ Hart², Kerry Atkinson¹, David J Munster², Alison M Rice¹

¹Biotherapy and ²Dendritic Cell Programs, Mater Medical Research Institute, South Brisbane, Queensland, Australia

Aim

Graft versus Host Disease (GVHD) is a major limitation of allogeneic haematopoietic stem cell transplantation (HSCT). Pre-transplant myeloablative conditioning (MAC) regimens controls malignancy, ablates host immune responses and facilitates haematopoietic reconstitution, but they are associated with significant treatment related mortality (TRM). Conditioning drives host dendritic cell (DC) and donor T-cell interactions resulting in GVHD. Reducing the intensity of the conditioning (RIC) regimen maintains anti-leukaemic activity and reduces TRM but the overall incidence of GVHD is unchanged. No clinically relevant in vivo murine models of RIC allogeneic HSCT exist, preventing studies into the mechanism of RIC-associated delayed-onset GVHD.

Methods

We developed two RIC HSCT models (full MHC mismatched (UBI-GFP/BL6 [H-2d]-BALB/c [H-2b] and MHC matched, minor histocompatibility mismatched (UBI-GFP/BL6 [H-2d]-BALB.B [H-2d]) that result in delayed onset GVHD. The development of these models has allowed the effect of RIC on chimerism and DC chimerism and subsequent GVHD to be investigated.

Results

In the fully MHC mismatched HSCT model, whilst there was no difference in overall survival between RIC and MAC HSCT recipients, RIC HSCT recipients required sacrifice for lethal GVHD significantly later ($d+23.11 \pm 2.88$, $n=9$) than MAC recipients ($d+6.6 \pm 0.1$, $n=23$; $p<0.000001$). In the MHC matched, miHA mismatched HSCT model, more RIC HSCT recipients survived until $d+90$ than MAC recipients ($p<0.001$) and those RIC recipients requiring sacrifice were taken significantly later ($d+60 \pm 7.9$, $n=11$) than their MAC counterparts ($d+23.04 \pm 1$ ($n=24$); $p<0.000001$). RIC activates and increases overall DC numbers post-transplantation without triggering early GVHD. Delayed onset GVHD in these RIC HSCT recipients is characterized by the persistence of host DC and delayed emergence of activated donor DC. Excess activated host DC in the BM of RIC mice was the only difference between RIC and MAC mice in the absence of donor cells. RIC induces lower levels of proinflammatory cytokines (TNF α and IL-1 β) during conditioning or early post transplant as compared with MAC.

Conclusions

In RIC HSCT recipients the excess activated host DC would be expected to cause earlier or more severe GVHD but the reverse was true suggesting that the pathogenesis of RIC-associated delayed onset GVHD is different to that seen in the MAC setting.

Tuesday 16 October

1030-1130

HSANZ Free Communications 5: Haematopoietic Stem Cell Transplantation I

Session sponsored by Schering-Plough Pty Ltd

Arena 1B

O74

1115

Cyclosporin, Methotrexate and Prednisolone for Graft-versus-host disease Prophylaxis in Allogeneic Peripheral Blood Progenitor Cell Transplants

Rosemary Hoyt¹, David Ritchie¹, Andrew Roberts¹, Lachlan MacGregor², David Curtis¹, Jeff Szer¹, Andrew Grigg¹.

¹*Department of Clinical Haematology & BMT Service, ² Clinical Epidemiology and Health Service Evaluation Unit, The Royal Melbourne Hospital, Parkville VIC 3050 Australia*

Unlike in allogeneic bone marrow transplants, the utility of graft versus host disease prophylaxis with cyclosporin, methotrexate and prednisolone (CSA/MTX/Pred) in allogeneic peripheral blood progenitor cell (PBPC) transplants is not well described. To determine the effectiveness of this regimen for prevention of acute GVHD, characteristics and transplant outcomes were compared in patients receiving CSA/MTX with or without prednisolone.

172 consecutive patients from September 1997 to 2006 receiving filgrastim mobilised PBPC allografts from either sibling or less than ideal donors were included. 107 patients received standard CSA, short course MTX and prednisolone, commencing on day+ 14 at 0.5mg/kg/day, increasing to 1mg/kg/day from day+21 to d+34 then gradually tapered and ceased by day +100; 65 patients received CSA/MTX alone. Data were collected regarding the cumulative incidence (CI) of acute GVHD, infection rates, transplant related mortality (TRM) and event free survival (EFS). The primary analysed outcome was the occurrence of grade II-IV acute GVHD and was assessed as the cumulative probability of acute GVHD. TRM and EFS were analysed using the Cox proportional hazards model. Identical supportive care regimens were given to both groups and treatment for GVHD was no different.

The CI of acute GVHD (grade II-IV) to day+100 in those receiving CSA/MTX/Pred was lower, 52% vs. 76%, $p < 0.01$. The onset of symptomatic GVHD requiring systemic treatment was delayed from a median of 41 days to 92 days post transplant. However, when CI was assessed to d+180, the incidence of acute GVHD became similar (74% vs. 78%). There was no difference between the two groups in rates of relapse, infections, severe acute GVHD or chronic GVHD. We conclude that the addition of prednisolone to CSA/MTX slows the rate of onset of acute GVHD in PBPC recipients but does not reduce GVHD incidence or diminish its severity. More effective prophylactic regimens are required.'

Tuesday 16 October

HSANZ Free Communications 6: Haematopoiesis

1030-1130

Meeting Room 6

075

Dissecting Myelopoiesis in Zebrafish

1030

Duncan Carradice, Luke Pase, Judith Layton, Graham Lieschke
Walter & Eliza Hall Institute, Melbourne, Victoria, Australia

The zebrafish (*Danio rerio*) has become an established model organism in which to study haematopoietic biology and disease processes. Zebrafish possess nucleated red cells, neutrophils, macrophages, thrombocytes, T and B cells, demonstrated to have high morphological, functional and genetic similarity to their human counterparts.

Zebrafish have several advantages over other vertebrate animal models eg. forward genetic screens using ethylnitrosourea (ENU) mutagenesis have the potential to recover mutants in genes involved in the pathway of interest and can be done on a scale and with an economy not possible in other vertebrates. Our laboratory has undertaken such a screen and recovered mutants with defects in mRNA expression of early and/or late (*pu.1+/-mpx*) myeloid lineage markers (myeloid failure mutants). Tools available for analysing haematopoiesis in zebrafish include transgenic lines expressing green fluorescent protein (GFP) under the control of promoters directing GFP expression to specific haematopoietic cell types (*scl*, *pu.1*, *mpx*, *lysozyme*, *gata1*, CD41). These allow in-vivo real time analysis of haematopoietic precursor migration, initiation and resolution of inflammation and thrombocyte function. FACS analysis of blood and kidney marrow enables sorting of haematopoietic cell lineages for further analysis (eg. morphology, gene expression etc.). Transient, reverse genetics techniques, such as over-expression or knockdown of a gene of interest are easily performed through microinjection of mRNA or morpholino antisense oligonucleotides into 1-4 cell embryos, respectively.

As example of this, the mutant *durif* has normal neutrophil numbers but is deficient in myeloperoxidase enzymic activity, modelling the most common neutrophil granule disorder of humans. The *marsanne* mutant has specific loss of the mature neutrophil marker *mpx* with normal expression of markers for haematopoietic precursors (*scl*) and red cells (*gata1*, *globin*). Positional cloning has defined a genetic interval of 300kb containing 13 genes; gene sequencing is underway. Cloning and characterisation of these mutants, such as *marsanne*, will lead to new understanding of the genetic control of myelopoiesis and may identify novel genes involved in human congenital neutropenia syndromes.

Tuesday 16 October
HSANZ Free Communications 6: Haematopoiesis

1030-1130
Meeting Room 6

O76

1045

Tracking Haematopoietic Stem Cells in a Living Organism

Enid Lam, Chris Hall, Phil Crosier, Kathy Crosier, Maria Vega Flores
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Aim

Runx1 is the most common target for translocation in acute leukemias and is essential for the development of haematopoietic stem cells (HSCs) during embryogenesis. The *Runx1* gene is transcribed from two promoters, P1 and P2, giving rise to a number of alternatively spliced transcripts important in the precise control of Runx1 levels during different stages of haematopoietic differentiation. Because Runx1 is both structurally and functionally conserved in humans and zebrafish, we have developed transgenic zebrafish lines using the *runx1* promoters tagged with EGFP, to track HSCs during development in a living organism.

Method

The zebrafish *runx1* promoters were cloned and used, with the *ToI2* transposon system, to generate transgenic EGFP lines. Lineage tracing was also performed using caged-fluorescent dextran. Images were captured by live fluorescent stereomicroscopy and confocal microscopy.

Result

The *runx1*P1 promoter was expressed at low levels in presumptive HSCs. In contrast, the *runx1*P2 transgenic strongly expressed EGFP in definitive blood precursors of the caudal haematopoietic tissue. In juveniles and adults, EGFP cells populated the thymus and kidney (the haematopoietic organ of adult zebrafish). Crossing the *runx1*P2 line with a *lysozymeC*-DsRED2 transgenic revealed co-expression in a subset of myelomonocytic cells.

Conclusion

These Runx1 transgenic lines are a new tool to study Runx1 regulation and function in normal and malignant haematopoiesis. The ability to visualise and isolate fluorescently labelled HSCs should contribute to further understanding of processes involved in stem cell development, maintenance and multi-lineage differentiation.

Tuesday 16 October
HSANZ Free Communications 6: Haematopoiesis
1030-1130
Meeting Room 6
O77
1100
A Global Role for Zebrafish Klf4 in Embryonic Erythropoiesis

 Melissa Gardiner¹, Milena Gongora¹, Sean Grimmond^{1,2}, Andrew Perkins^{1,2}
¹ *Institute for Molecular Biosciences, University of Queensland, Brisbane, Australia.*
² *The Australian Zebrafish Phenomics Facility, University of Queensland, Brisbane, Australia*

There are two waves of erythropoiesis, known as primitive and definitive waves in mammals and lower vertebrates including zebrafish. The founding member of the Kruppel-like factor (KLF) family of CACCC-box binding proteins, EKLF/Klf1, is essential for definitive erythropoiesis in mammals but only plays a minor role in primitive erythropoiesis. Morpholino knockdown experiments have shown a role for zebrafish klf4 in primitive erythropoiesis and hatching gland formation. In order to generate a global understanding of how klf4 might influence gene expression and differentiation, we have performed expression profiling of klf4 morphants, and then performed validation of many putative target genes by qRT-PCR and whole mount in situ hybridization. We found a critical role for klf4 in embryonic globin, heme synthesis and hatching gland gene expression. In contrast, there was an increase in expression of definitive hematopoietic specific genes such as larval globin genes, runx1 and c-myb from 24 hpf, suggesting a selective role for klf4 in primitive rather than definitive erythropoiesis. In addition, we show klf4 preferentially binds CACCC box elements in the primitive zebrafish γ -like globin gene promoters. These results have global implications for primitive erythroid gene regulation by KLF-CACCC box interactions.

Tuesday 16 October
HSANZ Free Communications 6: Haematopoiesis

1030-1130
Meeting Room 6

O78

1115

Indian Hedgehog is Essential for Definitive Haematopoiesis in the Fetal Liver

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¹ *Institute for Molecular Biosciences, University of Queensland, Brisbane, Australia.*

² *The Australian Stem Cell Centre, Monash University, Melbourne, Australia*

Indian hedgehog (Ihh) is a member of the hedgehog family of secreted proteins which play diverse roles in development. Members of this family have established roles in craniofacial development, long bone formation and spermatogenesis. Mammals have three hedgehog proteins which all act through the Patched receptor to activate smoothened and downstream zinc finger transcription factors of the Gli family. Ihh can induce differentiation of primitive red blood cells during primitive streak formation, and definitive red blood cells from CD34+ cord blood stem cells. Both Ihh and the Patched receptor are expressed in the fetal liver. In this report we Ihh knockout mice display a partially penetrant defect in fetal liver haematopoiesis characterised by severe anaemia and apoptosis of the fetal liver erythroid compartment. Primitive haematopoiesis is normal, so mutant embryos survive until ~E14. Components of the hedgehog signalling pathway are expressed in stromal, stem cell and progenitor cell components of the haematopoietic system. Lastly, fetal liver progenitor cells (CFU-e, BFU-e, and myeloid CFUs) are normal in Ihh null mice, suggesting a critical requirement for Ihh in the fetal liver stem cell niche.

Tuesday 16 October

1030-1130

HSANZ Free Communications 7: Myeloproliferative Disease

Meeting Room 8

Session sponsored by Bristol Myers Squibb

O79

1030

Residential Exposure to Electric Power Transmission Lines and Risk of Lymphoproliferative and Myeloproliferative Disorders: A Case-Control Study

Ray M Lowenthal¹, Deirdre Tuck¹ and Issy Bray²

¹*Department of Haematology/Oncology, Royal Hobart Hospital, Hobart, Tasmania, Australia; and* ²*Department of Social medicine, University of Bristol, Bristol, UK*

Aim

Studies have suggested an association between electromagnetic fields and childhood leukaemia. The aim of this study was to determine whether there is an increased risk of lymphoproliferative disorders (LPD) or myeloproliferative disorders (MPD) associated with residence within 300 m of high-voltage power lines.

Methods

Case-control study, using life-time residential history, of 854 patients diagnosed with LPD or MPD (including leukaemia, lymphoma and related conditions) aged 0-94 years comprising all cases diagnosed in Tasmania between 1972 and 1980. Controls were individually matched for sex and approximate age at the time of diagnosis.

Results

Compared with those who had always lived >300 m from a power line, those who had ever lived within 50 m had an odds ratio (OR) of 2.06 (95% confidence interval 0.87-4.91) for developing LPD or MPD (based on 768 adult case-control pairs); those who had lived between 50 and 300 m had an OR of 1.30 (0.88-1.91). Adults who had lived within 300 m of a power line during the first 15 years of life had a threefold increase in risk (OR 3.23; 1.26-8.29); those who had lived within the same distance aged 0-5 years had a fivefold increase in risk (OR 4.74; 0.98-22.9). These associations were strengthened when analyses were repeated for 201 pairs with entirely Tasmanian residential histories.

Conclusion

Although recognizing that this study has limitations, the results raise the possibility that prolonged residence close to high-voltage power lines, especially early in life, may increase the risk of the development of MPD and LPD later. (Internal Medicine Journal 2007 in press)

Tuesday 16 October

HSANZ Free Communications 7: Myeloproliferative Disease

Session sponsored by Bristol Myers Squibb

1030-1130

Meeting Room 8

O80

1045

Haematologic Parameters in Myeloproliferative Disorders According to JAK2-V617F Mutation Status

Z Kaplan, A Bell, A Kalff and A Tuckfield

Royal Melbourne Hospital, Haematology Department, Parkville, VIC

Background

The Janus Kinase/signal transducers and activators of transcription (JAK/STAT) pathway is pivotal in mediating signal transduction from growth factors in haematopoietic progenitors. JAK2 is a tyrosine kinase which facilitates intracellular signalling via interactions with membrane receptors for erythropoietin and thrombopoietin. The JAK2-V617F mutation results in constitutive activation. There is increasing evidence that the JAK2-V617F mutation has a causative role in the pathogenesis of myeloproliferative disorders and their clinical course.

Aim

To perform a single centre retrospective study evaluating the correlation between haematologic parameters and JAK2 positivity in essential thrombocythosis (ET) and myelofibrosis (MF) and to establish the frequency of JAK2-V617F mutation in ET, MF and polycythemia vera (PRV) in our institution.

Methods

The results of allele-specific polymerase chain reaction analysis for JAK2-V617F in 168 patients with a proven diagnosis of ET, MF or PRV was related to full blood count parameters at the time of diagnosis.

Results

JAK2-V617F was detected in 91% (50/55), 66% (57/83), and 50% (12/24) of patients with PRV, ET and MF respectively. As it pertains to ET, presence of the JAK2-V617F mutation was associated with a significantly higher haemoglobin ($145\text{g/dL} \pm 29$ vs $134\text{g/dL} \pm 34$ $p=0.007$), haematocrit (0.43 ± 0.080 vs 0.40 ± 0.095 $p=0.002$) and white cell count ($11.5 \times 10^9/\text{L} \pm 9.2$ vs $9.1 \times 10^9/\text{L} \pm 5.4$ $p=0.02$), there was however no demonstrable difference in platelet count ($897 \times 10^9/\text{L} \pm 564$ vs $884 \times 10^9/\text{L} \pm 684$ $p=0.85$). With regard to MF there was no discernable statistically significant difference imparted by JAK2-V617F on haematologic parameters. A trend towards a higher white cell was noted, but this failed to reach statistical significance.

Conclusion

JAK2-V617F invokes a differential haematologic phenotype in ET. Further, we aim to study the relationship between JAK2-V617F in myeloproliferative disorders and a predisposition towards haemorrhagic and/or thrombotic phenomena, leukemogenesis or transformation to secondary myelofibrosis.

Tuesday 16 October

1030-1130

HSANZ Free Communications 7: Myeloproliferative Disease

Meeting Room 8

Session sponsored by Bristol Myers Squibb

O81

1100

Pulsed ABL Kinase Inhibition with Dasatinib Achieve Equivalent Inhibition of Proliferation and Induction of Apoptosis as Continuous ABL Kinase Inhibition

Devendra K. Hiwase, Deborah White, Timothy Hughes

Institute of Medical and Veterinary Science, Adelaide, Australia

Dasatinib, SRC/ABL tyrosine kinase inhibitor is more potent than imatinib (IM). Dasatinib has a short half life (5 to 6 hrs), with near complete recovery of BCR-ABL kinase activity eight hours after drug administration. We hypothesised that pulsed kinase blockade might allow leukaemic cells to proliferate and survive to a greater extent than continuous blockade. In BCR-ABL+ve cell lines and mononuclear cells (MNC) from CML-CP patients, there is complete inhibition of ABL kinase activity as measured by the % of phosphorylated Crkl after 2 hours of dasatinib. We demonstrated that 8 hours of washout (after 8 hours of drug) allows complete reactivation of P-Crkl. Intermittent dasatinib (ID x 3 days) induces significant cell death and inhibits proliferation. Although there is trend of increased cell death in continuous dasatinib [CD] compared to ID, the difference was not significant. CD34⁺ and CFSE⁺ cells (CML-CP patients n = 5) were cultured in serum deprived media in presence of growth factors with/without dasatinib at 200 nMol [CD & ID]. After 72 hours, live cells and undivided cells were similar between two groups [Table I].

Conclusions

- 1) Dasatinib induces near complete inhibition of P-Crkl which is reversible within 8 hours of drug washout.
- 2) Intermittent dasatinib induces significant cell death and inhibits cell proliferation in BCR-ABL⁺ cell lines and CD34⁺ cells from CML-CP patients. Although there was trend of increase cell death in continuous arm, the difference was not significant.

We conclude that, once a day dosing will achieve equivalent inhibition of proliferation and induction of apoptosis as twice a day dosing, even though the kinase inhibition achieved is intermittent.

Table I: Comparison of cell survival and proliferation between continuous and intermittent dosing s

| Dasatinib concentration (nMol) | % live cells*(SD) | Fold change in undivided cells (SD) [§] | Proliferation index(SD) |
|--------------------------------|-------------------|--|-------------------------|
| 0 | 100 | 1 | 3.02 (0.758) |
| 200 ID | 38.04(3.17) | 3.04 (0.340) | 2.27 (0.63) |
| 200 CD | 30.13(4.85) | 3.64 (1.223) | 2.1(0.65) |

ID: intermittent dosing schedule of dasatinib

CD: continuous dosing schedule of dasatinib

*: % Live cells were normalised to "no drug control" in each patient.

§: undivided cells were normalised to 1 in "no drug control" group and then treatment conditions were compared with control in each patient.

SD: Standard deviation

Tuesday 16 October

1030-1130

HSANZ Free Communications 7: Myeloproliferative Disease

Meeting Room 8

Session sponsored by Bristol Myers Squibb

O82

1115

Patient-specific BCR-ABL Genomic DNA Can Be Detected in CML Patients in a Complete Molecular Response Defined by Quantitative RT-PCR

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Aim

After imatinib treatment for CML some patients have persistently undetectable BCR-ABL using real-time quantitative RT-PCR (RQ-PCR), a "complete molecular response" (CMR). However, such patients may relapse after imatinib withdrawal. DNA is more stable than RNA and is present irrespective of the level of RNA expression. We sought to determine if a patient-specific DNA PCR assay could detect BCR-ABL below the level of CMR.

Method

Patient-specific DNA PCR for BCR-ABL was developed for three patients enrolled in the ALLG study of imatinib withdrawal in patients in CMR for ≥ 2 years. The genomic BCR-ABL breakpoint was identified in presentation material using long template PCR with a forward primer in BCR and reverse primers in ABL. A nested TaqMan DNA PCR assay with patient-specific primers and probes spanning the breakpoint was then used to detect BCR-ABL in follow-up samples. Each PCR was performed in two independent experiments. There were two negative controls in each assay; no template, and DNA from another CML patient. DNA PCR results were compared with prior RQ-PCR results.

Results

To assess specificity each DNA assay was tested on 5 other CML patients. No non-specific amplification was detected. Each patient had three DNA samples between the time of the last positive RQ-PCR result and the time of ceasing imatinib. Patient#1 had no detectable BCR-ABL DNA. Patient#2 had a single positive DNA result after 10 months in CMR, and was subsequently negative. Patients #1 and #2 remain in CMR 10+ months after imatinib cessation. Patient #3 had detectable BCR-ABL DNA at all three timepoints, and had molecular relapse by RQ-PCR three months after imatinib cessation.

Conclusions

Nested DNA PCR for BCR-ABL is more sensitive than RQ-PCR in the three patients analysed to date and DNA PCR results correlated with remission/relapse status after cessation of imatinib therapy. This study is on-going.

Tuesday 16 October

HSANZ Free Communications 8: Novel Therapeutics

1030-1130

Meeting Room 9

O83

1030

The Histone Deacetylase Inhibitor LBH589 Induces Clinical Responses with Associated Alterations in Gene Expression Profiles in Cutaneous T Cell Lymphoma

Leigh Ellis¹, Yan Pan², Gordon Smyth³, Daniel George⁴, Chris McCormack^{1,2}, Roxanne Williams-Truax⁴, Peter Atadja⁵, Charlie Zhao⁵, Margaret Dugan⁶, Ken Culver⁶, Ricky Johnstone¹, Miles Prince¹

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Aim

LBH589 is a novel histone deacetylase inhibitor (HDACi) and pre-clinical studies have demonstrated that HDACi alter gene expression. Other HDACi have induced disease regression in cutaneous T-cell lymphoma (CTCL). We aim to assess safety and activity of LBH589 in CTCL as part of a larger Phase I study, and examined changes in tumor gene expression in the first 24 hours following oral LBH589.

Methods

Patients with CTCL were entered into the oral DLT dose level 30mg M,W,F cohort (n=1), the subsequent MTD dose level 20mg M,W, F weekly (n=9). LBH589 was continued until disease progression or unacceptable toxicity. Six patients had 3mm punch biopsies from CTCL-involved skin lesions at 0, 4, 8 and 24 hours after administration, which were subjected to gene expression profiling using Affymetrix U133 plus 2.0 GeneChips with 47,000 probesets. Alteration in gene expression patterns was confirmed by QRT-PCR of selected genes.

Results

10 patients are currently evaluable for response. 2 of the patients attained a complete response (CR), 4 attained a partial response (PR), 1 achieved stable disease (SD) with ongoing improvement, and 2 progressed on treatment (PD). (RR = 6/10; 60%) Microarray data on 6 patients demonstrated distinct gene expression response profiles over time with the majority of genes being repressed. Twenty three genes were commonly regulated by LBH589 in all patients tested.

Conclusions

LBH589 induces responses in CTCL patients. Preliminary microarray analyses of tumor samples have identified distinct gene expression profiles.

Tuesday 16 October
HSANZ Free Communications 8: Novel Therapeutics

1030-1130
Meeting Room 9

O84

1045

Repeat Treatment with Iodine-131-Rituximab Is Safe and Effective in Patients with Relapsed Indolent B-Cell Non-Hodgkin Lymphoma Who Had Previously Responded to Iodine-131-Rituximab

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²*University of Western Australia, Fremantle Hospital, Fremantle, Western Australia*

³*University of Melbourne, Victoria, Australia*

Aims

¹³¹I-rituximab achieves response rates of 76% in patients with relapsed indolent B-cell Non-Hodgkin lymphoma (NHL). Small series suggest re-treatment with murine anti-CD20 radio-immunotherapy (Bexxar™, Zevalin™) is safe and effective. Humanized antibodies have a longer half-life than murine antibodies, potentially prolonging bone marrow radio-isotope exposure, causing cumulative myelo-suppression on re-treatment. We retrospectively analysed data on patients who received a second ¹³¹I-rituximab at relapse.

Methods

Patients received two unlabeled doses of rituximab 375mg/m² and individualized ¹³¹I-rituximab doses administering 0.75cGy whole body exposure. Patients were monitored with weekly FBE's until 12 weeks post-therapy or recovery from nadir, and annual thyroid function tests. Severity and duration of cytopenia, development of myelodysplasia (MDS), acute myeloid leukaemia (AML) or hypothyroidism were noted. We compared response durations and toxicity following first and second treatments.

Results

Sixteen patients [follicular (15), mantle cell (1)] who previously responded to RIT were re-treated [median inter-treatment interval 19 months (9-54)] at PMCC and Fremantle between 1999-2007 with 14 responses [88%], nine CR's [56%] and 36% of all patients predicted to be progression-free at 12 months. Six pts had longer remissions with the second treatment than their first. Time to progression post second treatment (median 11 months) did not differ from the first treatment (median 14 months; P=0.89). Rates of Grade 3/4 haematologic toxicity were; neutropenia 29%, thrombocytopenia 27%, and did not differ from first treatment (both 25%). One patient required packed cells. Three patients have subsequently required thyroxine supplementation, but no cases of thyroid cancer have occurred. AML was diagnosed in one patient at 28 months, although retrospectively MDS may have preceded first RIT. No cases of MDS were seen.

Conclusions

Re-treatment with ¹³¹I-rituximab is an effective and safe option for patients who have responded previously. Durable responses are not restricted to those who had a favourable response to initial treatment.

Tuesday 16 October

HSANZ Free Communications 8: Novel Therapeutics

1030-1130

Meeting Room 9

O85

p38^{MAPK} Inhibitors as Therapeutic Agents for Pre-B ALL

1100

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Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common childhood malignancy. Although responsive to chemotherapy and radiation, disease relapses occur in about 25% of children and 60% of adults, indicating that new approaches for treatment are required. Bone marrow stromal cells are a rich source of adhesion molecules and growth factors that promote leukemic cell survival *in vitro*. p38^{MAPK}, a stress activated protein kinase, normally associated with apoptosis is activated by chemotherapy or radiation. We and others have shown that p38^{MAPK} signaling can promote ALL cell proliferation and p38^{MAPK} inhibitors reduce stromal cytokine production. Overall, the role for p38^{MAPK} signaling in maintaining ALL cell growth and survival needs more clarification.

Aim

To determine the role for p38^{MAPK} signaling in growth regulation of leukemic cells and elucidate if p38^{MAPK} inhibitors (SB203580 and SB220025) can enhance chemotherapy-induced cytotoxicity *in vitro*.

Results

SB203580 reduced proliferation without affecting cell survival in 5 stroma-supported cultures of primary ALL cells. Similar results were obtained using SB220025, while cell survival was inhibited in one case with SB220025. In contrast the proliferation and survival of the stroma-independent ALL cell line, NALM6 was unaffected by SB203580. Treatment of ALL cells with cytotoxic agents Vincristine or Dexamethasone activated p38^{MAPK}, and this was inhibited by pre-treatment with SB203580 and SB220025. SB203580 enhanced anti-proliferative effects of Vincristine, Dexamethasone, Etoposide and Doxorubicin without increasing drug cytotoxicity in NALM6. In primary cases however, p38^{MAPK} inhibitors enhanced both cytotoxic and anti-proliferative effects of Vincristine and 1Gy radiation.

Conclusion

Bone marrow stromal cells support leukemia cell proliferation by enhancing signaling through the p38^{MAPK} pathway. Furthermore, p38^{MAPK} inhibitors sensitize ALL cells to chemotherapy or radiation-induced cell death by blocking stress-induced activation of p38^{MAPK}. There is potential for p38^{MAPK} inhibitors to be used in conjunction with current therapy for more effective treatment of ALL.

Tuesday 16 October
HSANZ Free Communications 8: Novel Therapeutics

1030-1130
Meeting Room 9

O86

1115

The R-ITP 1000 Study: A Multicentre, Single Arm, Open Label Study to Evaluate the Efficacy and Safety of 1000mg Fixed Dose of Rituximab on Day One and Fifteen Among Patients with Refractory, Relapsing or Chronic Idiopathic Thrombocytopenic Purpura

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Rituximab is a chimeric monoclonal antibody that was designed for the targeted elimination of B-cells, currently approved for the treatment of Non-Hodgkin Lymphoma (NHL) and Rheumatoid Arthritis (RA). The role of auto-reactive B-cells in the pathogenesis of Idiopathic Thrombocytopenic Purpura (ITP) has led to a number of clinical studies to investigate the potential benefit of rituximab in this condition.

Five prospective studies involving 142 adult patients with relapsed/refractory chronic ITP treated with 375 mg/m² rituximab weekly for four consecutive weeks have been published to date. These trials reported an overall response rate ranging from 50 to 75%, with a median time to response and median response duration (among complete responders) of 6 weeks and greater than 6 months, respectively. These studies also reported that rituximab was well tolerated. The existing evidence therefore suggests that rituximab is a viable option for the treatment of patients with refractory/relapsing ITP. It is not clear however whether the NHL-based dosing regimen is optimal in a non-malignant condition.

A new fixed dosing regimen of rituximab, 1000 mg administered on days 1 and 15, has been utilised in the treatment of RA. It was shown to be effective at achieving rapid (within 2 weeks) and prolonged (at least 6 months) B-cell depletion and reducing disease activity (measured by the American College of Rheumatologists criteria), with a safety profile similar to that reported in NHL studies.

The R-ITP 1000 study is the first prospective study to utilise this fixed dosing regimen in refractory or relapsed ITP. The overall objective is to document the efficacy and safety of this treatment strategy. Clinical responses will also be correlated with lymphocyte depletion, rituximab concentrations and Fc gamma receptor polymorphisms.

This study has recently been initiated and 106 patients are expected to be enrolled over a twelve months period at 15 participating Australian centres. The current study progress will be presented.

Tuesday 16 October

ASTH Free Communications 2: Clinical Studies/Thrombosis

1030-1130

Central Room C

O87

1030

Rapid, Safe, Effective and Convenient Warfarin Reversal Procedure, for Elective Surgical Procedures, Using Low Dose Intravenous Vitamin K

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³. *Royal Hobart Hospital, Hobart, Tasmania*

Background

The optimal management strategy for temporary warfarin reversal preceding surgery has not yet been established. Current guidelines recommend discontinuing warfarin 4-5 days prior, aiming for an INR <1.5. Patients may receive parenteral bridging anticoagulation, based on their risk of thromboembolism (TE). An alternative method involves administering low dose intravenous vitamin K (vit K IV) prior to the procedure. This is rapid and convenient, however, has not been thoroughly assessed for routine practice.

Patients/method

We undertook a prospective cohort study, at the Royal Hobart Hospital between January 2006 - June 2007, to assess the safety, efficacy and convenience of vit K IV, for short term reversal of warfarin. Patients on warfarin undergoing elective surgery received 3mg of vit K IV, between 12-24 hours prior to the procedure. The patient's INR was checked one hour pre-procedure. Patients were monitored post-operatively for 6 weeks. Patients at high TE risk received therapeutic LMWH post-operatively, until reestablishment of a therapeutic INR. Outcome measures included: adverse reactions to vit K; INR values; incidence of bleeding and thrombosis peri-procedure during follow-up; time to achieve therapeutic anticoagulation with rewarfarinisation.

Results

104 patients participated with median age 72 years. Major indications for anticoagulation included: atrial fibrillation (41%), prosthetic valve (23%), TE (22%). No patient suffered an adverse reaction to vit K. 97/104 achieved a pre-procedure INR ≤ 1.5 (93%), all achieved INR ≤ 1.7. Four patients had procedure-associated major bleeding – all these procedures had an inherently high bleeding risk. No patient suffered TE during 6 weeks follow-up. Of the 91 with completed data, the median days to re-establish a therapeutic INR was 5 (Range: 2-20 days).

Conclusion

Low-dose intravenous vitamin K appears to be safe and effective for the temporary reversal of warfarin, preoperatively. Re-establishment time of therapeutic INR was not prolonged. Bleeding and thrombotic risks were not increased. This method of reversal is rapid, convenient, cost-effective and avoids bridging anticoagulation.

Tuesday 16 October

ASTH Free Communications 2: Clinical Studies/Thrombosis

1030-1130

Central Room C

O88

1045

Fibrin Generation is Not Suppressed in Patients Warfarinised for Venous Thromboembolism (VTE)

Jennifer L Curnow^{1,2}, Marie-Christine Morel-Kopp^{1,2} and Christopher M Ward^{1,2}

¹Northern Blood Research Centre, University of Sydney, ²Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, Sydney, NSW, Australia

Warfarin therapy for VTE may be complicated by bleeding or recurrent thrombosis even when the INR is in the target range (2.0-3.0). We postulated that the OHP, a global coagulation assay, would show suppression of fibrin generation with a therapeutic INR. It may also identify individuals with increased risk of bleeding or thromboembolism.

116 patients taking warfarin for VTE were tested at least 6 weeks after their acute event. For the OHP, thrombin (0.03 U/ml) was used to trigger fibrin generation in platelet poor plasma (PPP) with rt-PA (300 ng/ml) added to initiate fibrinolysis. INR values, fibrinogen, factor VIIIc and d-dimer levels were assayed. OHP results were compared with reference intervals determined in a reference population of 200 healthy Australians. Pearson correlations were calculated.

The distribution of patients according to INR was 30% (INR 1.5-1.99), 60% (INR 2-3) and 10% (INR >3). Only 3 patients with INR > 2 showed the expected suppression of fibrin generation. 66% had OHP parameters in the normal range and 31% had hypercoagulable parameters despite a therapeutic INR. Fibrinogen was significantly elevated ($p<0.001$) in the hypercoagulable group but FVIIIc was not significantly different between the groups.

There is significant discrepancy between INR values and OHP parameters in patients taking warfarin for VTE. The majority do not show suppression of fibrin generation, *in vitro*, despite a therapeutic INR. INR values were significantly correlated with the delay in onset of fibrin generation ($p=0.001$) and its rate of formation ($p<0.001$) but not with other OHP parameters. We found a non significant trend toward elevated d-dimer in warfarinised patients with hypercoagulable OHP results (0.14 vs 0.32; $p=0.367$). We hypothesize that patients with increased fibrin generation, whilst on warfarin, may be at increased risk of recurrent VTE after warfarin cessation. We are collecting longitudinal data in these patients to further test our hypothesis.

Tuesday 16 October

ASTH Free Communications 2: Clinical Studies/Thrombosis

1030-1130

Central Room C

O89

1100-1115

PreVent – An Electronic VTE Risk Assessment Tool and Decision Support in Acute and Subacute Inpatients

Marita Reed¹, Jo Bourke¹, Claire Passlow², Philip Campbell³, Michael Vagg⁴, Neil Orford⁵, Simon Tomlinson⁶, Tony Weaver⁷

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Aim

Venous thromboembolism (VTE) is a significant problem for hospitalised patients, potentially resulting in serious illness and risk of death. Despite evidence from randomised controlled trials supporting the role of thromboprophylaxis in hospitalised patients, it is clear that VTE risk assessment and implementation of thromboprophylaxis remains underutilised, particularly in medical patients. We aimed to increase the rate of VTE risk assessment and appropriate thromboprophylaxis clinical decision-making across Barwon Health using an electronic tool in hospitalised patients.

Method

We developed an electronic VTE risk assessment and prophylaxis decision support tool that was linked to the patient management and pathology reporting information systems. The tool used existing computer-generated unit-based patient lists to identify the risk status of admitted patients and enabled outcome reports to be automatically generated and distributed via email to unit heads.

Results

Following implementation of this protocol, the proportion of patients who had a VTE risk assessment completed increased from 51.58% in August 2006 to 85.9% in March 2007. Appropriate thromboprophylaxis use increased from 58.18% to 89.25% during the same period across acute and subacute services.

Conclusion

The implementation of an electronic VTE risk assessment and thromboprophylaxis decision support tool, when integrated into existing information management systems across acute and subacute health services, changed clinical behaviour and improved compliance with evidence-based thromboprophylaxis guidelines.

Tuesday 16 October

ASTH Free Communications 2: Clinical Studies/Thrombosis

1030-1130

Central Room C

O90

1115

Low-Dose Aspirin for Secondary Prophylaxis of Vein Thrombosis (the ASPIRE Study) Baseline Characteristics and Event Rates

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Introduction

The optimal antithrombotic management of patients beyond first episode of unprovoked venous thromboembolism (VTE) is uncertain.

Methods

The ASPIRE study compares low-dose aspirin (100mg daily) with placebo for prevention of recurrent vein thrombosis in patients completing standard anticoagulation for first unprovoked VTE. The primary efficacy endpoint is symptomatic recurrent VTE, secondary endpoints are arterial vascular events (MI, stroke, CV death), vascular mortality, and all-cause mortality. The primary safety endpoint is major bleeding. Patients are followed for an average of 3 years. The ASPIRE and WARFASA studies are combined in a planned prospective meta-analysis (INSPIRE study).

Results

427 patients had been randomized into ASPIRE from Australian and New Zealand centres by June 2007: 51% female; median age 57 yrs (IQR 46-69); qualifying events proximal DVT alone 206 (48%), PE alone 144 (34%), both 68 (16%); duration initial anticoagulation 26% <6 months, 58% 6 to 9 months, 16% > 9 months; 41% BMI >30 ("obese") and 36% BMI of 25-30 ("overweight"). A significantly higher proportion of females compared with males had a BMI > 30 (48% vs. 31%; $p < 0.001$). In 560 patient-years follow up the unadjusted annualized events rates are: VTE 5.7% (95% CI 3.8-8.1); MI 0.5% (95% CI 0.1-1.6); Stroke 0.2% (95% CI 0-1.0); any death 1.4% (95% CI 0.6-2.8); and all CVD events (VTE, MI, stroke and CVD death) 6.4% (95% CI 4.5-9.0). The annualized rate of major bleeding was 0.7% (95% CI 0.2-1.9). Males had a higher risk of recurrent VTE than females (8.5% per annum vs. 2.6% per annum, $p = 0.006$).

Conclusion

Three quarters of included patients are either overweight or obese. Event rates (total and VTE) are significantly higher among men than women. Maintenance of a 6% annual primary event rate and an increase in recruitment from existing and international sites are needed to meet the study's primary objective.

Tuesday 16 October**1030-1130****Diagnostic Laboratory Science: Complex Phenotypes in the Haemoglobinopathies****Meeting Room 5****Case Presentations and Panel Discussion**

Ronald Trent, Swee Lay Thien, Don Bowden & Anne Gilbert

Complex Phenotypes in the HaemoglobinopathiesSwee Lay Thein¹ and Ronald Trent²¹ King's College Hospital London UK and ² Royal Prince Alfred Hospital, Camperdown, NSW Australia

Haemoglobinopathies have always provided a challenge to a range of health professionals because their phenotypes (i.e. haematologic findings as well as clinical presentations) are variable. This reflects the underlying molecular pathology being a mix of many different mutations and the interplay of a number of genes. Complex phenotypes have always existed, so why the interest now? Two developments explain this renewed interest. (1) Of particular relevance to Australia, are the emerging risk groups. Earlier teaching that thalassaemia is a disease of the Mediterranean changed as risk populations migrated from different countries including many parts of Asia, Eastern Europe, the Middle East and now Africa. With these came new gene combinations and new phenotypes. Hb Variants, which comprise the other component of the haemoglobinopathies, were once uncommon with only Hb Lepore (e.g. Italian populations) and a few very rare examples found. Today, HbE and more recently HbS and their various interactions with thalassaemia are important to detect and manage. (2) The second development is technologic, and associated with this comes the medico-legal consequences of missing something that is detectable. Family studies were once the predominant way to pursue complex phenotypes and without them little could be done outside the research environment. Today, this is no longer a barrier as genes can be sequenced to piece the puzzle together. In this session, we will present a number of interesting haemoglobinopathies and illustrate what are reasonable approaches in their investigation, and where more information is needed to avoid unnecessary work and raising anxiety in patients.

Tuesday 16 October
ANZSBT: Ruth Sanger Oration

1130-1230
Arena 1B

Blood Laws

“The regulation of blood as a therapeutic product”

Richard Pembrey AM

The use of blood to rescue people from death has a long history. The story begins with experiments transfusing blood from animals or human donors. The outcomes were usually unfavourable. Only with the recognition of blood groups a little more than 100 years ago did blood transfusion become a respectable medical intervention. Discoveries and advances in development of anticoagulant solutions, storage and separation of blood components saw the establishment of a blood transfusion industry and the emergence of blood components and products as a ready to use therapeutic product by clinicians.

The blood products proved so effective in supporting advances in surgery and medicine that benefits were seen to greatly outweigh the risk of adverse outcomes. That was until the appearance of AIDS. The blood industry was a potent vehicle for the transmission of the human immunodeficiency virus. The effects demanded oversight of the industry by a regulator to ensure safety, quality and efficacy.

This will be the story of my personal involvement in a part of this process, from clinician involved in unregulated collections, testing and transfusions to oversight of the manufacture of blood components and products by a regulatory agency, and a reflection on the lesson learned that will apply equally to the emerging use of human cells and tissues in the treatment of disease.

Tuesday 16 October
HSANZ BMTSANZ Symposium: BMT

1330-1500
Arena 1B

1330

Regulatory T Cells from Murine Models to the Clinic

Robert S Negrin
Stanford University Medical Center, Stanford, CA, USA

Graft-versus-host disease (GVHD) is a major obstacle to success in the clinical application of allogeneic hematopoietic cell transplantation (HCT). Several strategies have been attempted to reduce GVHD risk. A particularly attractive approach has been to utilize regulatory mechanisms designed to modulate immune reactions in an attempt to control the over exuberant immune reaction characterized by GVHD. We have explored regulatory T cell populations in an effort to reduce GVHD risk since well characterized CD4⁺CD25⁺ FoxP3⁺ T_{reg} have been shown to suppress the mixed lymphocyte reaction. Using highly purified (>98%) populations of T_{reg} in murine models of allogeneic HCT, we have demonstrated that the addition of T_{reg} in a ratio of 1:1 to conventional CD4⁺ and CD8⁺ T cells (T_{con}) results in control of GVHD yet with maintenance of a GVT response. Using a novel bioluminescent based imaging strategy with luciferase (*luc*) expressing cells derived from transgenic *luc* expressing animals we have visualized the trafficking and survival of both T_{con} and T_{reg} cells following allogeneic HCT. Both home to secondary lymph node sites and proliferate. Interestingly the T_{reg} persist for approximately 4-6 weeks without causing GVHD whereas the T_{con} continue to proliferate and cause GVHD resulting in animal lethality. The addition of T_{reg} 48 hours prior to T_{con} results in the ability to reduce the total dose of T_{reg} 2-5 fold. In models of an infectious challenge with murine CMV, we have demonstrated that T_{reg} block the GVHD immunosuppression induced damage of the thymus and lymph nodes characterized by GVHD following T_{con} administration and allows these animals to control CMV infection much more efficiently. We are developing strategies to explore the use of T_{reg} in the clinic which will be discussed.

Tuesday 16 October
HSANZ BMTSANZ Symposium: BMT

1330-1500
Arena 1B

1415

Who Gets an Allograft for Lymphoma?

Anthony Goldstone

North London Cancer Network, University College Hospital, London, UK

Conventional allogeneic transplant has been thought of as a “last resort” for lymphoma patients who have failed all previous therapies. In relation to this, it had been the habit five to fifteen years ago to offer up occasional heavily pre-treated patients to conventional ablative sibling allograft for relapsed lymphoma. Many of these patients still had refractory disease. The result was series and registry data with a very high treatment related mortality, sometimes above 50%, accompanied by considerable relapse because of refractoriness of the disease which made these attempts an unattractive way forward and clearly not a benefit to patients.

In the last seven or eight years, it has become apparent that the allogeneic effect does exist for some lymphomas, and can be harnessed with so called “mini” transplants producing much less treatment related mortality and yet still produce an allogeneic effect in previously heavily treated patients. Not only that, subsequent donor lymphocyte infusion for progression can in some circumstances produce a clear benefit and return to remission. It is also apparent that in the “mini” setting this allogeneic effect can be useful even in unrelated donor transplants where the results can be clinically acceptable from the point of view of treatment related mortality albeit with a greater level of TRM than that seen in sibling transplants.

This presentation will review the data for the group and the indications now used for the selection of patients.

Tuesday 16 October

ANZSBT ASTH Symposium: Transfusion and Coagulation

1330-1500

Central Room A

1330

Coagulation and Ventricular Assist Devices

John F Fraser

The Critical Care Research Group, The Prince Charles Hospital, Brisbane, Queensland, Australia

Rapid advances in the medical treatment of heart failure have improved quality of life and life expectancy. However end-stage heart failure poses immense challenges to the clinician. Cardiac transplantation has been the mainstay of treatment for a small group of patients who can no longer be managed effectively with medication, surgery or pacemaker techniques. Unfortunately the limited donor pool restricts the number of patients who can be offered transplantation. Mechanical devices for circulatory support have been proposed as a bridge for patients to successful transplantation and more ambitiously are now proposed as a long term alternative to transplantation. Cost of these devices is substantial as are the problems related to the biological-mechanical interphase of the blood supply and chronic long term extracorporeal circuitry. The haematological issues associated with ventricular assist device placement can be divided into early and late. Massive transfusion requirements are not uncommon in the immediate post-operative phase of VAD placement. As with all cardiopulmonary bypass, thrombocytopenia, relative hypothermia and hypocalcaemia occur. Patients who requires ventricular assist device urgently are frequently prescribed adjuvant pharmacotherapy associated with cardiac disease such as glycoprotein IIb-IIIa antagonists, coumarins, unfractionated and low molecular weight Heparins. Cardiac dysfunction per se may also initiate a bleeding diathesis due to hepatic congestion and renal dysfunction in association with right and left ventricular dysfunction. 60% of patients with ventricular assist device replacement will require return to operating theatre for excess of uncontrollable bleeding. This excess bleeding has financial costs as well as an association with increased mortality and morbidity. Transfusion of platelets has been shown to make cross matching of potential hearts much more difficult. In the late phase post VAD placement, excess coagulation is the more common problem. The extracorporeal circuitry is colonised by a neointima consisting of activated inflammatory cells as well as haemopoietic stem cells. These cells are known to produce high levels of tissue factor. In association with this increased level of activated cells there is increased B cell activity and pro-inflammatory cytokine formation.

The improved understanding of the coagulation abnormalities in the early and late phase post ventricular assist device will undoubtedly improve the outcome of the post ventricular assist device patient. The speaker will address both use of VADs and potential areas of research which may optimise control of coagulation disorders in this rapidly growing area.

Tuesday 16 October
ANZSBT ASTH Symposium: Transfusion and Coagulation

1330-1500
Central Room A

1400

The Use of Recombinant Activated Factor VII in Australia and New Zealand – Evidence from the Haemostasis Registry

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Introduction

Recombinant activated factor VII (rFVIIa, marketed under the brand name NovoSeven®) is approved for the treatment of spontaneous and surgical bleeding in patients with haemophilia A or B and with antibodies to either factor VIII or factor IX. Recently rFVIIa has been used increasingly often for indications outside the approved areas, particularly in cardiac surgery, trauma and other critical bleeding episodes. Use in these areas remains controversial. Monash University Department of Epidemiology and Preventive Medicine has established the Haemostasis Registry (with an unrestricted educational grant from Novo Nordisk Pharmaceuticals Pty Ltd) to collect data on the use of rFVIIa in non-haemophilic patients with critical bleeding throughout Australia and New Zealand.

Data Received to Date

Sixty-six hospitals have completed the process of joining the Haemostasis Registry, obtaining ethics approval and are now able to submit data to the Registry. A further eight hospitals have agreed to participate in the Registry project and are awaiting ethics approval. It is anticipated that all the major users of rFVIIa in Australia and New Zealand will contribute to the registry. As at 1 August 2007, 1335 cases had been received.

Variables showing a univariate association (p -value less than 0.1) with the outcome variables were entered into the multivariate analyses. Haematocrit and Haemoglobin levels were not included as they showed a strong co-linearity with Units RBC (Spearman correlation, $p < 0.001$).

Response: Units RBC ($p=0.034$) and pH ($p<0.001$) were both found to be associated with response when taking into account place of administration, INR, APTT, age, temperature, fibrinogen and platelet levels. For each additional unit of RBC transfused, patients were less likely to respond (OR 0.98, 95% CI 0.97-0.99). For each additional unit increase in pH, patients were 52 times more likely to respond (OR 52, 95%CI 17-162).

Mortality: pH ($p<0.001$) and Platelet Level ($p<0.001$) were found to be associated with mortality when taking into account temperature, fibrinogen level, APTT and RBC units. For each additional unit increase in pH, patients were 104 times less likely to die (OR 104, 95% CI 35-303). For each additional unit increase in platelet level, patients were 1.007 times more likely to survive (OR 1.007, 95% CI 1.004-1.009).

Conclusions

Although randomized controlled trials are important in establishing the safety and efficacy of new treatments, they do not replace the need for registries, especially for treatments where clinicians believe that withholding treatment may be unethical because of potential life threatening consequences. This problem is made more difficult where there are a wide range of applications and where cases of specific indications are relatively rare. As more data becomes available, the Haemostasis Registry data will help to elucidate the safety and efficacy of rFVIIa and provide important feedback to doctors and hospitals.

Tuesday 16 October

ANZSBT ASTH Symposium: Transfusion and Coagulation

1330-1500

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1430

Pro-Inflammatory Endothelial Activation and Organ Failure from Stored Blood

Christopher C Silliman

Bonfils Blood Center and Department of Pediatrics, University of Colorado School of Medicine, Denver, CO, USA

Traumatically injured patients are prone to developing early multiple organ failure (MOF) 72 hours post-injury dependent upon a number of clinical factors, including: patient age, the injury severity score (ISS), and the mechanism of injury. In patients with intermediate ISS (15-35) the most robust predictor of MOF was a transfusion requirement of >6 units of PRBCs over the first 12 hours; moreover, longer PRBC storage of these PRBC units also predicted MOF. Because MOF is PMN-mediated, begins with acute lung injury, and is the result of at least two events, we **hypothesize** that stored PRBCs caused pro-inflammatory activation of the vascular endothelium leading to PMN sequestration.

Method

PRBCs were collected from whole blood and 50% (by weight) was pre-storage leukoreduced (LR) and 50% was left as an unmodified control. Primary pulmonary human microvascular endothelial cells (HMVECs) were incubated with heat-treated (to obviate complement and fibrinogen) plasma from fresh (day 1) or stored (day 42, the day of outdate) NLR-PRBCs and LR-PRBCs for 6 hours and intercellular adhesion molecule-1 (ICAM-1) surface expression, chemokine release, and PMN adherence were measured via flow cytometry, ELISA or MPO assays, respectively.

Results

Day 42 LR-PRBCs plasma [10-40%] and NLR-PRBC plasma [20-40%] caused significant increases in ICAM-1 surface expression, 1.5 ± 0.2 to 2.4 ± 0.8 -fold, versus plasma from day 1 PRBCs from the identical units ($p < 0.05$). Plasma from stored but not fresh NLR- and LR-PRBCs also caused significant synthesis and release of growth related oncogene- α , interleukin-8 and epithelial neutrophil activating protein 78 versus day 1 NLR- and LR-PRBC plasma ($p < 0.05$). This increased surface expression of ICAM-1 and release of chemokines induced significant PMN adherence (1.5 ± 0.1 - 3.5 ± 0.6 -fold) compared to day 1 NLR- and LR-PRBC plasma ($p < 0.05$). We **conclude** that stored blood, irrespective of leukocyte contamination, causes pro-inflammatory activation of HMVECs resulting in PMN adherence possibly predisposing injured patients to MOF.

Tuesday 16 October
HSANZ Symposium: Haematopoiesis

1530-1630
Arena 1B

1530

Exploiting an Understanding of Foetal Haemoglobin Control in Adults for the Therapeutic Application of β globin Gene Disorders

Swee Lay Thein

King's College London School of Medicine and King's College Hospital, London UK

The β hemoglobinopathies (β thalassaemia and sickle cell disease, SCD) remain an enormous clinical problem globally affecting more than 300,000 births per year. The prevalence of these disorders is increasing at particularly alarming rates; in many countries SCD has become the most common and fastest growing serious genetic disease. Both disorders display a remarkable but difficult to predict variability in clinical severity. A major ameliorating factor is the innate ability to produce fetal haemoglobin (HbF, $\alpha_2\gamma_2$). HbF and F cells (erythrocytes that contain measurable amounts of HbF) levels vary considerably not only in patients with β haemoglobin disorders, but also in healthy normal adults. The majority of the quantitative variation of HbF is heritable ($h^2=0.89$), but the genetic aetiology is complex, with several contributing quantitative trait loci (QTLs). *Cis*-acting variants (major being *Xmn1*-G γ in the β globin locus on chromosome 11p15) accounts for up to 30% of the variance but >50% of the HbF variance results from genetic factors not linked to the β globin locus. *Trans*-acting QTLs include those on 6q, 8q, 2p and Xp. Three major genetic loci (*Xmn1*-G γ , 6q and 2p QTLs) are associated with 50% of HbF variance in European Caucasians. Approaches on dissecting the genetic architecture of HbF / F cell trait are discussed. Identification of these genetic contributors to HbF / F cell levels should allow an improved prediction of one's ability to produce HbF, and ultimately a better understanding of the pathways and mechanisms of HbF control, providing new targets in therapeutic approaches for HbF augmentation.

Tuesday 16 October
HSANZ Symposium: Haematopoiesis

1530-1630
Arena 1B

1605

Ikaros Drives Human Haemoglobin Switching by Facilitating Active Chromatin Hub Formation

Janelle Keys¹, Michael Tallack¹, Ye Zhan², Peter Papathanasiou³, Christopher Goodnow³, Karin Gaensler⁴, Merlin Crossley⁵, Job Dekker² & Andrew Perkins^{1,6}

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The human β globin locus consists of an upstream locus control region (LCR) and five functional genes arranged sequentially in the order of their expression during development: 5'- ϵ - γ^G - γ^A - δ - β 3'. Haemoglobin switching entails the successive recruitment of these genes into an active chromatin hub (ACH). Although much is known about the cis elements and transcription factors involved in globin gene regulation, less is known about ACH formation. Here we show that the transcription factor Ikaros plays an essential role in both the formation of the β -globin ACH, and in haemoglobin switching. In Plastic mice, where the DNA-binding region of Ikaros is disrupted by a point mutation, there is concomitant down-regulation of human β -globin, and up-regulation of γ -globin gene expression. We show Ikaros and its family member Eos, bind to critical cis elements in the β -globin locus. Furthermore our data suggest that Ikaros facilitates long range looping between the LCR and a region upstream of the δ -globin gene. This study provides new insights into the mechanism of adult stage-specific assembly of the β -globin ACH. In addition the findings could lead to the development of novel drugs to reactivate HbF in adults with β -thalassaemia.

Tuesday 16 October
ANZSBT Symposium: Governance of Blood/ANZTPA

1530-1630
Central Room A

1530

Regulation of Blood and Blood Products – Integration Within Appropriate Governance

Albert Farrugia

Blood and Tissues Unit, Therapeutic Goods Administration, Australian Government, Canberra, Australia

Blood components have been regulated since 2000 using concepts which are akin to those used in pharmaceutical manufacture. Because of this, blood processing which is closer to the clinical than the manufacturing interface has been exempt from the Therapeutic Goods Act (1989). This has been most notable in the case of autologous and directed donations. In addition, some hospital based blood processing can be captured by the Act if done outside the context of individual patient use. The development of a staged regulatory system for all biologicals, generated as a result of the currently delayed regulatory authority for Australia and New Zealand, lends itself to the incorporation of clinically governed processes into a system which will ensure appropriate standards for safety and efficacy throughout the blood system. This presentation will review progress towards this system and discuss some of the remaining hurdles.

Tuesday 16 October

ANZSBT Symposium: Governance of Blood/ANZTPA

1530-1630

Central Room A

1605

Role of Accrediting Bodies in Transfusion Practice

Darlene Hennessey

Abstract not received at time of going to print

Tuesday 16 October
ASTH Symposium: Oral Anticoagulation Control

1530-1630
Central Room C

1530

Oral Anticoagulant Control in Lupus Anticoagulant Patients

Ian Mackie

Haemostasis Research Unit, Haematology Department, University College London, UK

The incidence of thrombosis in patients with antiphospholipid syndrome (APS) is increased and some reports have recommended a higher intensity of warfarin treatment. However, later studies have shown that higher INR values give no reduction in thrombosis incidence while the bleeding risk is greater. A target INR of 2.5 is generally considered appropriate for venous thrombosis. Other studies have suggested higher target ranges because the PT/INR system may be falsely increased by interference of lupus anticoagulant (LA) with the phospholipid component of the PT reagent, particularly where recombinant tissue factor is employed. The majority of patients (>95%) with APS have a normal PT in the absence of other coagulopathies. When the PT is prolonged, it is often due to hypoprothrombinaemia, resulting from high affinity antibodies and immune depletion. These antibodies differ from those commonly seen in APS, which have low affinity and cause no prothrombin depletion. Certain reagents are more sensitive to LA and indeed have been utilised in LA detection systems. However, the perceived between reagent differences in INR in LA patients mostly disappear when instrument and reagent specific INR/ISI calibration is performed. All patients should have a baseline PT and where this is elevated, alternative PT reagents may be employed. In the rare patients with significant prolongation of the baseline PT and difficulty in establishing the true degree of anticoagulation, amidolytic factor X assays may be helpful. It is possible that some of these patients may also have antibodies directed against factor VII or tissue factor. Activation marker assays (D-dimer, TAT, F1.2) have been employed by some, to assess ongoing thrombin generation and efficacy of warfarin prophylaxis, but there are no well controlled studies. The use of point of care devices for INR in APS should be performed with caution. Some devices are very sensitive to LA and most manufacturers list this as a specific exclusion to their use.

Tuesday 16 October
ASTH Symposium: Oral Anticoagulation Control

1530-1630
Central Room C

1600

Point of Care INR

Michael Ray
Pathology Queensland, Brisbane, Australia

Objective

Point of Care (POC) monitoring of oral anticoagulant therapy is providing improved outcomes for both selected patients who can adequately self-monitor and those patients who do not have easy access to routine laboratory testing. This presentation will describe the home monitor, the CoaguChek and its successor, the CoaguChek XS and the use of the latter for patients requiring ventricular assist devices (VAD) and who are able to go home. It will also describe the implementation of INR testing across Queensland on a different class of point-of-care analyser, the i-STAT 1.

Methods

Each of the three analysers was evaluated individually at different times as they became commercially available. Patients being routinely monitored for oral anticoagulant therapy at our hospital were selected. The phlebotomists were trained in the use of the instruments and performed POC INR determinations at the bedside using fingerprick blood at the same time as drawing venous samples for laboratory INR testing.

Results

The CoaguChek was considered suitably accurate for use in the hospital's paediatric ward, but only up to an INR of 3.0. It was in use successfully for 5 years.

The improved agreement of the CoaguChek XS with the laboratory results made it possible for VAD patients to self-monitor at home.

The excellent accuracy of the i-STAT INR led to its routine use in 40 sites around Queensland.

Discussion

Pathology Queensland are responsible for quality assurance of POC instruments, training of medical and nursing staff in their use, and storage of the data. The i-STAT has facilitated this process. Group co-ordinating laboratories in the larger hospitals manage the procurement, quality assurance and distribution of test cartridges. In the last year 2830 INRs were performed on the i-STAT throughout Queensland in a timely and controlled manner.

Tuesday 16 October

1530-1630

Diagnostic Laboratory Science: New Laboratory Tests and Techniques

Meeting Room 5

1530

Monitoring Human Cytomegalovirus-Specific Cellular Immunity Using QuantiFERON®-CMV

Tania Crough¹, Susan Walker¹, Stephen Jones² and Rajiv Khanna¹

1 Australian Centre for Vaccine Development, Queensland Institute of Medical Research, Brisbane, Australia

2 Cellestis Inc., Melbourne, Australia

We have longitudinally analyzed HCMV-specific CD8⁺ T-cell responses in a cohort of heart and/or lung transplant patients. The detection of active virus replication correlated with a reduced IFN- γ expression by HCMV-specific CD8⁺ T-cells and an up-regulation of CD38 expression on the surface of virus-specific CD8⁺ T-cells, although there was no significant change in the number of virus-specific cells. Most importantly, analysis of IFN- γ production by virus-specific CD8⁺ T-cells in patients with HCMV disease suggested that disease was associated with a reduction in CD8⁺ T-cell responsiveness to both structural and immediate-early antigens. Based on these results, we have developed a novel diagnostic technology to monitor the HCMV-specific CD8⁺ T-cell responses referred to as QuantiFERON®-CMV. Evaluation of QuantiFERON®-CMV in healthy individuals and solid organ transplant patients revealed that this technology was at least as sensitive and with some HCMV epitopes more sensitive than the ELISPOT for detecting ex vivo IFN- γ . Results from QuantiFERON®-CMV assays showed 97% (36/37 individuals) agreement with the anti-HCMV serology test in healthy individuals. Taken together, this study indicates a pivotal role for IFN- γ producing virus-specific CD8⁺ T-cells in protecting transplant patients from HCMV disease, and this protection is dependent on a broadly focused T cell responses rather than a response directed towards any one single antigen. Furthermore, QuantiFERON®-CMV is a simple, reproducible and reliable test for the detection of IFN- γ in response to HCMV CD8⁺ T-cell epitopes, and may be a valuable diagnostic test for the detection of HCMV infection and as a useful clinical tool for monitoring the immune response in immunosuppressed patients during therapy.

Tuesday 16 October

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Diagnostic Laboratory Science: New Laboratory Tests and Techniques

Meeting Room 5

1545

Serum Free Light Chain Assay

Jill Tate

Chemical Pathology, Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia

Clinical Utility

Measurement of serum free light chains (FLC) is useful for the diagnosis and monitoring of monoclonal light chain diseases in particular non-secretory and light chain myeloma, and AL amyloidosis. The FLC assay may also have a role in monitoring intact immunoglobulin myeloma where disease response to treatment may be detected earlier by serum FLC than standard protein electrophoresis due to the shorter half-life of kappa and lambda light chains compared with the intact immunoglobulin molecule. From the point of view of clinical validation of FLC, the major studies have been performed in the diagnostic, not monitoring, setting.

Analytical Issues

The use of polyclonal anti-human FLC antibodies in immunoassay methods for measurement of monoclonal free light chains raises the question of adequate specificity and affinity to bind to individual monoclonal FLC. Given there may be differences in immunoreactivity between monoclonal and polyclonal proteins, there is the possibility of either overestimation or underestimation of serum FLC. At diagnosis and particularly, for long-term monitoring of monoclonal light-chain disease, reproducible and accurate assay performance is required. Laboratory staff and clinicians should be aware of the potential for non-reactivity of individual monoclonal FLC, the effect that dilution has on FLC measurement, and an appreciation of the impact of assay imprecision on result interpretation. Some monoclonal light chains (particularly kappa FLC) do not dilute in a linear fashion and may be underestimated in the absence of additional off-line dilutions and assay imprecision, especially with different lots of FLC reagent, may have a significant effect on changes in the FLC concentration and the calculated kappa/lambda ratio. These issues, if not adequately appreciated, have the potential to mislead clinical diagnosis and assessment of response to therapy.

Tuesday 16 October

1530-1630

Diagnostic Laboratory Science: New Laboratory Tests and Techniques

Meeting Room 5

1600

Rationale for International Harmonisation of BCR-ABL Monitoring by RT-PCR

David Ross, Timothy Hughes, Susan Branford

Institute of Medical & Veterinary Science, Adelaide, SA, Australia

Real-time quantitative reverse transcriptase PCR for BCR-ABL (RQ-PCR) is used to monitor treatment of CML patients. A major molecular response (MMR) has prognostic significance and guides therapeutic decisions. Various RQ-PCR methods are used, and the value representing MMR varies between labs. To overcome this problem an international reporting scale (IS) was proposed, standardizing the absolute value representing MMR as 0.10%. Conversion to the IS uses laboratory (lab)-specific conversion factors (CFs). Thirty-four labs from 13 countries sent 615 patient samples to the reference lab in Adelaide to determine their specific CF. RQ-PCR methods varied by control gene (ABL n=17, BCR n=12, GUSB n=4, other n=4), primers and probes, instruments, and standards. Each lab-specific CF was calculated from the bias of patient BCR-ABL values between the originating lab and the reference lab using the method of Bland and Altman. CFs were determined for 33 methods; 4 methods failed CF determination due to inconsistencies in the bias or insufficient number of samples. CFs were validated using subsequent sets of patient samples sent to the reference lab. The specific CF remained valid for each method. After conversion the mean bias between the reference values and the originating lab values was negligible. Over 95% of values were within ± 5 -fold of the reference value; 68% of values were within ± 2 -fold. Only 0.6% of values were ± 10 -fold. In contrast, prior to conversion the limits of agreement indicated that 95% of values were within ± 13 -fold of the reference value. Importantly after conversion the concordance of values in the range representing MMR was 87% (154/178 samples). Ideally, conversion to the IS should be achieved using certified reference material, which is currently under development. Standardized molecular monitoring will enable more consistent application of treatment guidelines, and aid interpretation of clinical trial data.

Tuesday 16 October

1530-1630

Diagnostic Laboratory Science: New Laboratory Tests and Techniques

Meeting Room 5

1615

Implementation of Quantitative BCR-ABL Molecular Monitoring

David Fairbairn

Molecular Genetics Laboratory, Pathology Queensland – Central Laboratory, Royal Brisbane Hospital, Herston Hospitals Campus, Herston QLD 4029

Chronic myeloid leukaemia (CML) is one of the most frequently diagnosed forms of leukaemia. Real-time quantitative polymerase chain reaction (RQ-PCR) methodology has become widely used for the detection of *BCR-ABL1* fusion transcripts caused as a consequence of the t(9;22)(q34;q11) translocation. Regular molecular monitoring using RQ-PCR is valuable for assessing the patient's response to therapy, minimum residual disease and the early detection of drug resistant subclones.

The development of standardised RQ-PCR protocols and comparable reporting from different pathology laboratories is clearly desirable for clinicians. We have implemented an RQ-PCR protocol for the detection of *BCR-ABL1* transcripts that was developed by the Institute of Medical and Veterinary Science (Adelaide) using the Europe Against Cancer Program primer and TaqMan probe sets. Our experiences of establishing and performing the RQ-PCR test at Pathology Queensland – Central Laboratory over the last 12 months will be presented.

Notes: