Anti-D quantitation working party

Graeme Woodfield, Tanya Davison, Brian Fisher and Julie Watt

Abstract not available at time of printing

Improving appropriateness of blood transfusion in the 21st century

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Although blood has never been safer, fatal and non-fatal complications still occur. Data from the literature indicates that red cell & plasma components are frequently used inappropriately with reports of 20 60% non-indicated transfusions. Such practice needlessly places recipients at risk. Attempts to modify physician transfusion practice have frequently been unsuccessful. Recently, the concept of academic detailing has demonstrated impressive gains in improved drug prescribing practices. Academic detailing uses educational outreach targeted at opinion leaders. The educational messages are clear, concise, well-founded and repeated frequently. Objective: This study evaluated the usefulness of this concept in transfusion practice. The Harvard Transfusion Intervention Project used university-based educational outreach to reach opinion leaders within hospitals. This was a multi-center, randomized controlled study of surgeons and medical specialists that addressed RBC transfusion practices. Baseline transfusion data were collected for 1 year prior to intervention. 101 physicians were visited by a transfusion expert for 20-30 minutes. The one-on-one sessions were interactive. The key messages were 1) the 30% transfusion trigger is dead ; 2) the complications of transfusion are not negligible; and 3) indications for transfusion. Six months after intervention, medical record audits demonstrated a 40% decrease in non-compliant transfusions (p=.006) in surgeons, but not medical specialists. Post-intervention, surgeons performed transfusions when hematocrits were 2% lower than before the intervention (p=.04). Conclusion: Academic detailing is a successful method for changing transfusion behavior.

Transfusing the difficult patient: antibody identification and blood provision

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The IBGRL receives blood samples from around the world for investigation of unusual and rare blood group antigens and antibodies. Difficulties may arise in the investigation itself, which is often of a complex nature, and in the provision of compatible blood for transfusion. Apart from investigation of patients with a rare blood group the largest single group of patients referred to us are those with sickle cell anaemia.

In general a high incidence antigen is defined as one present in >97% of a population and incidence may differ depending on racial background. It is therefore useful to know the ethnic background of a patient. Antibody identification involves many factors: incidence and mode of serological reactivity, strength of reaction with different cells and sensitivity or resistance to proteolytic enzyme and chemical modification of test cells can all be useful clues. Rare specificities may be difficult to identify, especially if additional antibodies are present ie anti c, Fy^a, Jk^b and investigations can be laborious and time consuming causing problems in urgent cases. Compatible donors for patients with rare phenotypes may be rare worldwide.

Sickle cell disease patients may be chronically transfused and the frequency of alloimmunisation is relatively high. Antibody mixtures can change over a period of time and serological monitoring is needed on a regular basis. Prevention of alloimmunisation may be achieved by phenotyping the patient at time of diagnosis and using only closely matched donor units. Similarities of phenotype within this patient population means that the same donor population is constantly targeted and this may be especially difficult when there is a racially discordant donor-recipient pool.

Quality Assurance in Transfusion Serology

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The RCPA Transfusion Serology QAP was established in 1969 with 100 participants and one survey distributed that year. The program has evolved over the years to three modules with upto six surveys/module per year and over 300 participants in Australia, New Zealand and other international sites. Compared to overseas Transfusion QAPs, the program is designed to emulate the practice in a hospital/pathology transfusion service with analysis of clerical errors of name and unit number recognition. Assessment is qualitative with comparison with referees and comparison to accepted guidelines for performance. Educational ungraded exercises are issued to challenge the wide range of laboratories participating. Performance improvements have been noted in many technical areas over the years. The standardisation of techniques and use of computerisation in laboratories challenges the QAP to simulate normal laboratory practice.

The last five years of surveys (1995 2000) will be reviewed and examines both the technical and procedural changes that have taken place during the past five years. Surveys, which usually take the form of wet exercises, and educational exercises, which may take the form of wet or dry exercises, are sent out to participants every two months. Although the primary purpose of the surveys is their role in quality assurance, a significant emphasis is placed on the educational value of the exercises provided and as far as possible they are designed to represent real life clinical situations. In addition, technical surveys are carried out every two to three years. These are in the form of a questionnaire, which addresses most aspects of the participants laboratory practice

The RCPA transfusion Serology QAP first investigated the possibility of establishing an FMH survey in 1995. Between 1996 and 2000 several educational exercises were sent out to participating laboratories and in 2001 an FMH Survey was distributed for the first time. Preliminary results showed large variations in CV of the Kleihauer test for estimating FMH while the Flow Cytometry test employed by a small number of laboratories showed much less variation. This presentation examines the development of the FMH survey and presents some possible ways of improving the Kleihauer test with reference to other surveys. It is hoped that the FMH survey will become an integral part of the laboratory s external Quality Assurance.

Antibody Quantitation: The Queensland Experience

T Davison
Australian Red Cross Blood Service, Brisbane

The ARCBS-QLD Red Cell Reference Laboratory quantitates the level of anti-D and anti-c in patient samples, to assist clinicians with the management of antenatal patients at risk of haemolytic disease of the newborn. In response to the shortage of Rh(D) Immunoglobulin in Australia, ARCBS-QLD initiated a program to increase the concentration and volume of plasma containing anti-D we provide to CSL Bioplasma. The Red Cell Reference Laboratory assess individual plasma donations for high or low titre allocation and provides a quantitative value of the amount of anti-D sent to CSL Bioplasma. This laboratory is also responsible for monitoring the red cell serology of a number of donors selected for our Anti-D Boosting Program. The procedure, quality assurance and performance indicators for antibody quantitation will be discussed.

Supernatants from Stored Red Blood Cell Concentrates Activate Neutrophils: Influence of Leucocyte Burden

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Immunomodulatory mediators released by leucocytes present in fresh blood products are thought to contribute to adverse reactions experienced by some transfusion recipients. Although a number of studies have reported the levels of various immunomodulatory factors in blood products, minimal in vitro functional data is available. The aim of this study was to investigate the neutrophil activating potential of supernatants from stored red blood cell concentrates (RBC). Three types of RBC were investigated: 1) routine hard spun RBC (Group 1); 2) buffy coat-depleted RBC (Group 2); and 3) leucocyte-filtered RBC (Group 3). Level of supernatant IL-8, induction of the adhesion molecule CD11b on neutrophils by exposure to RBC supernatants and neutrophil chemotactic responses to RBC supernatants were assessed.

RBC were prepared, according to standard procedures, from whole blood collected into packs containing CPD anti-coagulant and Adsol solution (Baxter). Group 2 RBC were collected into Optipacs and processed by the Optipress (Baxter). Group 3 RBC were leucocyte filtered by a log 4-removal filter (BPF4, Pall). All RBC were stored at 4 C for 42 days. Samples, aseptically collected on day 1 and each week thereafter, were centrifuged to obtain the supernatant and stored at 70 C. Supernatant IL-8 levels were determined by a sandwich ELISA (R&D Systems). Induction of neutrophil CD11b by RBC supernatant was determined by incubating fresh whole blood with RBC supernatants, or fMLP as control, followed by staining with FITC-labeled anti-CD11b and flow cytometric analysis. Chemotaxis experiments were performed in ChemoTx microplates (Neuro Probe) in which fluorescence-stained neutrophils were separated from RBC supernatants or controls by a 3 mm pore-size filter. After incubation, neutrophil migration was determined by a fluorescence plate reader (Victor^2 1420 Counter, Wallac).

Results are presented in the Table below.

RBC Groups	Leucocytes / RBC Unit (x 10^6)	Supernatant IL-8 (pg / ml) Day 1	Day 42	% increase CD11b (Day 42 Day 1)	Chemotaxis (% max) Day 1	Day 42
Group 1 (n=7)	2452–596	14–33	264–114	34–14	3–1	52–19
Group 2 (n=6)	473–267	0	0	0	nt	9–2
Group 3	0.3–0.1	0	0	0	nt	0

Mean–SD nt=not tested

These results demonstrate that immunomodulatory factors capable of stimulating heterologous neutrophils accumulate during storage of RBC. These effects were virtually eliminated by pre-storage reduction of leucocytes and hence provide evidence for a rationale for a benefit of leucocyte removal from RBC.

Development and Implementation of a Comprehensive Haemovigilance System in a Major General Hospital

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Quality management of safe transfusion within hospitals requires surveillance of all stages of the process from request generation to post transfusion. In most hospitals , recognition and recording of adverse transfusion related events (ATRE) is mainly confined to clinical reactions. At this hospital a haemovigilance programme (HP) was established and designed to record adverse events at any point in the chain of activity culminating in blood and blood product transfusion.

Implementation of the HP was preceded by an educational programme for the various levels of hospital staff involved. Cooperation and compliance was achieved by 1) Adopting an anonymous / no blame reporting protocol, 2) Registering the programme as a quality activity to achieve protection of the data generated from access under the Freedom of Information act , 3) Designing a simple and distinctive report form. ATRE were categorised as 1) Sample collection errors, 2) Transport / courier incidents, 3) Laboratory incidents 4) Blood / product handling incidents, 5) Clinical reactions.

Following an educational campaign, the HP was implemented in May 2000. During the 10 months to the end of March 2001, there were a total of 235 incidents reported. These comprised 69 sample collection errors, 18 transport / courier incidents, 34 laboratory incidents, 52 blood / product handling incidents and 62 clinical reactions.

The data has identified areas of practise which can be targeted for improvement and provided a basis for developing improved systems for the delivery of safe transfusion within the hospital.

Development of monoclonal antibodies for the detection of the infectious agent of Creutzfeldt-Jakob disease (CJD) in blood

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The normal form of the Prion Protein (PrP^c) is predominantly expressed in brain tissue, and is thought to play a role in regulating resistance to oxidative stress. The infectious form of the Prion Protein, PrP^{sc}, has the same amino acid sequence as PrP^c, however it has a different secondary and tertiary structure. There is an urgent need to develop a reagent for the screening of blood to identify individuals carrying PrP^{sc}. ARCBS is involved in a collaborative study, with the Department of Pathology, University of Melbourne, which aims to generate monoclonal antibodies (Mabs) using peptides from the Prion Protein. These Mabs will be vital reagents in the development of diagnostic assays for the identification of PrP^{sc} in blood and blood products.

PrP knockout mice were immunised with a peptide (PrP 90-120-linked to Keyhole Limpet Haemocyanin) from the Prion Protein that is most likely to undergo transformation during its conversion from the non-infectious to infectious form. Spleen cells from the immunised mice were fused with myeloma cells and the hybrid cells screened (by ELISA) for their ability to recognise the peptide from the Prion Protein. Supernatants from the most reactive hybrid cells were sent to the University of Melbourne for immunohistochemical testing on CJD-infected human brain tissue. A number of the supernatants were strongly positive with CJD-positive but not normal brain tissue. The antibody 3F4, which is considered the world gold standard, was used as the positive control.

Western blot analysis has also shown that some of the ARCBS antibodies recognise brain material from CJD-infected individuals and they detect three forms of PrP^{sc} representing the unglycosylated, monoglycosylated and diglycosylated species. The profile obtained on brain tissue is consistent with that seen with a classical case of CJD. While further studies are required to characterise the antibodies and to establish stable antibody-producing cell lines, the objective is to develop a specific high affinity antibody for high through-put screening, that will detect the infectious Prion in Blood.

Antibodies to variant MNS (Miltenberger) antigens detected in Australia and Asia: are some cases of anaemia of prematurity undiagnosed haemolytic disease of the newborn?

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In ethnic groups from southern China and SE Asia, 60% of antibodies detected in pretransfusion testing are to variant antigens of the MNS system (vMNS), sometimes collectively designated anti-Mi(a) . In Australia, Mi(a) -positive screening cells are not used and the prevalence of antibodies to these antigens is unknown. The first aim of this study was to investigate the prevalence of antibodies to vMNS antigens MNS7, MNS10/Mur, MNS26/Hop and MNS20/34-Hil/MINY on the basis of reactions with captured biotinylated peptides in an ELISA. The second was to compare the pattern of reactions with these antigens for individual Mi(a) -positive sera. Antibody activity was quantified by measurement of absorbance following incubation with HRP-protein-G and then substrate. A result was scored as positive when the ratio of absorbance (test-peptide/control-no peptide) was >2 for a 1:20 dilution of serum. Antibody was detected in four of 112 Australian blood donors (3.8%), five of 124 antenatal patients (4%) and three of 215 multitransfused patients (1.4%) using a screening ELISA in which the four biotinylated peptides were mixed. The positive sera were subsequently tested with the individual peptides. ELISA-positive sera from Caucasians reacted with the MNS10/Mur peptide only. Anti-MNS10/Mur was detected in serum from the mother of a neonate with jaundice of prematurity . In one case, in an Australian of Philippine extraction, anti-MNS10 and anti-MNS7 were both detected. During an international exchange in Taiwan, 150 sera from patients and blood donors that agglutinated Mi(a) positive red cells were investigated. Antibodies with specific reaction(s) in the vMNS-peptide-ELISA were detected in 64 sera (43%). Anti-MNS7 (41 sera, 27%), anti-MNS10 (33 sera, 22%) and anti-MNS26 (15 sera, 10%) were detected more frequently than anti- MNS20/34 (3 sera, 2%). In 16 sera (11%) anti-MNS7 and anti-MNS10 were both detected. For 18 sera (12%), the reaction pattern was non-specific equivalent high absorbance for test and control possibly reflecting antibody binding to streptavidin or the plastic surface. For 68 sera that were Mi(a) -agglutination-positive vMNS-peptide ELISA-negative (46%), failure to detect the antibody may have been because the titre was <20 or the anti-Mi(a) may have reacted with carbohydrate rather than peptide antigens. In summary, while vMNS-peptide-ELISA-reactive sera from Australian Caucasians reacted with only one peptide (MNS10/Mur), for Taiwanese people there were several patterns. The relative clinical significance of anti-Mi(a) that react with a single antigen (eg. anti-MNS10/Mur) compared those that react with several antigens (eg. anti-MNS7 with anti-MNS10), is a question for further investigation.

Approaches to protecting patients against variant CJD; prevention better than cure?

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The discovery that BSE can be transmitted to humans as variant CJD (vCJD) has had a profound effect on blood services world-wide, but especially in the UK. Measures introduced in Scotland (population 5 100 000; ~10% of the UK) to avoid the unknown risk of vCJD transmission by blood cost at GB£9.9 million annually, plus an initial GB£1.3 million capital, for importation of plasma for fractionation and universal leukocyte depletion of blood components. Further precautionary measures such as importing FFP as well as tissues are being considered. Obtaining red cells and platelets from outside the UK is not a realistic option, other than perhaps for neonates. The above measures, aimed at increasing the safety of blood components, would add significantly to the cost and the possibility of extending donor exclusions to previously transfused persons will add further costs and reduce the blood supply by 8 20%. An estimated cost for Scotland of implementing all the new precautions would be GB£14 million per annum a total of GB£24 million for all the precautions. Other measures might be considered that concentrate on reducing the risk from local blood supplies. Serious measures to minimise transfusion have yet to be taken, although this is not due to lack of interest by blood services. The Scottish National Blood Transfusion Service Effective Use of Blood group has introduced a transfusion nurse led clinical effectiveness (CE) programme in a small number of hospitals and preliminary results have demonstrated that such a scheme can lead to appropriate minimisation of transfusion. A CE programme consisting of audit, education, guideline/protocol development and the promotion of alternative therapies (but not funding of e.g. EPO) could be implemented throughout Scotland for GB£2 million annually. Such measures to minimise the avoidable use of blood will impact on all transfusion associated risks, and not only vCJD. While the risk of vCJD remains unknown, and could still be zero, it would appear most appropriate to take all measures that would minimise or avoid blood use while not compromising clinical outcome. Although various initiatives have been proposed to reduce blood usage, only a major change in the awareness of clinicians and patients to the risks of transfusions is likely to lead to the required changes, and this will require the concerted effort of the whole health service from Government down, if it is to be successful.

Finding the Balance Between Innovation and Compliance

Adrian Oates
Quality Director, CSL Bioplasma

Over the last 15 years, the development of biological products has been subject to increased regulation in Australia. Prior to 1989, clinical trials were not required for biological products and GMP inspections were infrequent. The Therapeutic Goods Act was introduced in 1989 to adopt the European regulator standards for biological products. Extensive biochemical/chemical characterization and clinical trials are now required to demonstrate safety and efficacy of new products. Data from these must then be submitted to the Therapeutic Goods Administration (TGA) for approval to supply the product on the Australian market. In addition to drug legislation, good practice and quality system concepts have evolved including codes of Good Clinical Practice (GCP), Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP).

Over the last six years, CSL Bioplasma has developed a whole new range of products. To achieve this, an effective communication process was required. Internally, full-time project managers focused the efforts of cross-functional groups to meet time, quality and cost requirements. Quarterly senior management reviews ensure accountability. Externally, CSL Bioplasma s Regulatory Affairs group communicated progress updates to the TGA.

Once the product development process was complete, the transfer, scale-up and routine manufacture of products into the production environment involved a different set of guidelines managed under the Good Manufacturing Practice s Code of Regulations. GMP regulations aim to ensure the pharmaceutical quality of the product and therefore regulate manufacturing personnel, facilities and equipment, documentation, manufacture, quality control, product complaints, recall procedures and self inspections. These elements are evaluated both through the TGA evaluation phase and by TGA GMP audits. Once a product has been granted marketing approval, there are additional requirements for adverse experience reporting (pharmacovigilance) and monitoring of the product stability whilst in the field.

The development and maintenance costs of plasma derived products have increased significantly in recent times and the incentive to further improve processes are further challenged today by measures to ensure the viral and prion safety of plasma. These issues are being resolved through constant, sensible, scientifically based discussions with the TGA and other interested parties.

In summary, innovation is possible in a GMP compliant environment but this requires careful attention to regulatory standards and quality systems. Effective communication with the regulator assists in an outcome that both protects and benefits the Australian public.

Custom sterile and GMP compliance

Annette Grundy
Baxter Healthcare

Abstract not available at time of printing

Regulatory Strategies for Class 111 medical devices the artificial heart

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The VentrAssist Implatable Rotary Blood Pump is a novel left ventricular assist device being developed as destination therapy for patients with end stage heart failure.

The VentrAssist IRBP is designed to sit within the abdomen, immediately below the diaphragm or within the thoracic cavity. It is joined to the circulatory system by an inflow cannula that is attached to the left ventricle and an outflow cannula that is attached to the ascending aorta.

The VentrAssist IRBP impeller is suspended hydronamically in blood within the sealed pump chamber. The hydronamic suspension is achieved by using thick impeller blades with tapered edges. Unlike other rotary pumps that utilise pivot or thrust bearings and shafts to suspend the impeller, the design of the VentrAssist IRBP minimises blood damage by reducing regions of high shear and fluid residence time. The impeller is driven electromagnetically by an interaction between rare earth magnets embedded within the impeller and currents in copper coil windings located in the void between the housing and the shroud of the pump. The impeller is driven by a controller that may be powered by the mains power supply or batteries.

The VentrAssist IRBP is considered an active implantable Class 111 medical device, since it is a life-supporting device that may potentially cause illness or injury to the patient. In order to gain approval of this device clinical safety and effectiveness needs to be demonstrated through a series of bench and animal testing followed by clinical trials. What do the regulatory bodies such as the TGA and FDA require to satisfy them?

Red cell transfusion guide lines

Adele Green

Abstract not available at time of printing

Pattern of cryoprecipitate usage at the Alfred hospital over a 6 month period

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The Alfred hospital is a quaternary care hospital and as such our department supports many acutely ill patients as well as the Adult Haemophilia service of Victoria (including other bleeding disorders).

A review of our cryoprecipitate usage over a six-month period was undertaken as part of our quality management. The aim was to asses which patients were receiving cryoprecipitate and what indications there were for this usage.

All patients who received pooled cryoprecipitate were examined. The patients with severe Von Willebrands disease formed one group, massive transfusion patients contributed to the second group. The latter group was reviewed for their INR, APTT, Fibrinogen and Platelet count and what other products they received as well as the number of bags of cryoprecipitate infused.

Cryoprecipitate is prepared by thawing and pooling each donation of 30 ml. Over a six-month period 1475 bags of cryoprecipitate were pooled. 738 bags (50%) went to 3 patients in the Von Willebrands group while 737 bags (50%) went to 68 patients in the second group.

This second group comprised acutely ill, bleeding patients from our Trauma and Surgical units. In this group the median Fibrinogen prior to administration of cryoprecipitate was 1.1g/L range 0.1 5.5g/L, the INR 2.8 range 1.1 11.8, APTT 90.0 seconds range 25.6-300 seconds and platelet count $118x10^9$/L, range 17 $427x10^9$/L

All coagulation tests were performed on a STAGO STA coagulation analyser, which allows simultaneous testing of all three parameters. The fibrinogen is measured by the Von Claus method with an electromagnetic clot endpoint that is accurate to levels of 0.2 g/L.

Transfusion of cryoprecipitate is appropriate for patients with Von Willebrands disease and may also be used for correction of microvascular bleeding in massively transfused patients with fibrinogen levels below 0.8 1.0 g/L. The number of units of blood products given to these patients ranged from 10 to 156 units (not including the cryoprecipitate).

As a designated trauma centre we expect to receive patients who will require massive transfusion from time to time. We believe that we are assisted by the ability to determine the fibrinogen level accurately and in a timely manner allowing the clinicians to act on these results. We must also be mindful that the new platelet configuration supplied from the Australian Red Cross Blood Transfusion service, with pooled platelets suspended in T-Sol removes a hitherto unconsidered supply of fibrinogen, Von Willebrands factor and Factor VIII to the acutely bleeding patient.

Massive Acute Transfusion Episodes in a Patient with Clinically Significant Antibodies

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Mrs HS is a 59-year-old patient with renal disease who presented with acute gastro-intestinal bleeding. Blood grouping and antibody screening found her to be A2 RhD Positive with anti-S, anti-c and anti-A1 (active at 37 C). Twenty O R1R1 S- units of crossmatch compatible blood were transfused but her condition continued to deteriorate and immediate surgery was undertaken. As the previous transfusions had used all available O R_1R_1 S units in South Australia, it was decided that incompatible R_1R_1 units (presumably S+) were to be transfused during acute massive bleeding episodes, leaving any compatible R_1R_1 units (S) reserved for post-operative bleeding. Five incompatible units were transfused intra-operatively and 17 compatible units post-operatively. Her condition deteriorated again and a further six O R_1R_1 S+ units were transfused.

Over the ensuing days the DAT became positive and anti-S was eluted. A further 9 units of O R_1R_1, S blood were transfused over the following two weeks. Several weeks later the DAT was again positive and anti-c and anti-E were eluted. The patient was subsequently discharged and has since had many admissions for dialysis with only an occasional transfusion.

This patient, with multiple antibodies and acute bleeding presented a major challenge to the transfusion service. The only solution, within the timeframe, was to utilise incompatible units of blood during acute bleeding episodes, and to transfuse antigen negative units when the bleeding rate decreased. Based on published data it was considered that the anti-S was less likely to cause significant complications, and that Rh compatible units would be given at all times. The transfusion laboratory could not always confirm these typings in the time available. Anti-E and anti-c were subsequently eluted and it would appear likely that one or more units were in fact c positive and/or E positive.

When a patient has clinically significant antibodies it is standard transfusion practice to provide antigen negative blood. For alloimmunised patients with multiple antibodies requiring massive transfusions, this can prove to be very difficult, and occasionally impossible. Consultation between the laboratory scientific and medical staff and clinicians is essential to both provide the best available transfusion options and to ensure appropriate post-transfusion monitoring.

Appropriate Selection of Uncrossmatched Blood for Emergency Transfusion

Tony Greenfield
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Transfusion of uncrossmatched blood in the tertiary (level 3) trauma department at Liverpool Hospital is not uncommon and has been a relatively safe practice. An on-site supply of O Rh(D)Negative blood for females and O Rh(D) Positive blood for males has been readily available and commonly utilised. Group specific blood if possible is provided by the laboratory after the patient blood group is confirmed. Group specific blood adds a level of risk to emergency transfusion and offers no other advantage than the compelling need to conserve group O blood. To estimate the risk of a patient having a clinically significant antibody when transfusing uncrossmatched blood, a retrospective study of patients screened at Liverpool Hospital was undertaken. The appropriateness of providing uncrossmatched blood, and in particular O Rh(D)Positive blood to male patients, was considered.

Overall 1.7% of hospital patients were found with potentially clinically significant antibodies. The incidence of clinically significant antibodies was strongly influenced by clinical specialty and age . Only 2.3% of Emergency Department patients (1.8% for Trauma) compared with 6 to 7% of Renal or Haematology/Oncology patients, had clinically significant antibodies. Overall only 0.6% of patients under 30 years of age, compared with 3% of patients more than 60 years of age, had clinically significant antibodies. The incidence of clinically significant antibodies in Emergency Department patients under 30 years of age was also only 0.6%. Some basic knowledge of the patient therefore permits an estimation of risk associated with transfusing uncrossmatched blood.

The age and sex distribution of trauma patients in the Emergency Department is strongly biased towards young males. This group is unlikely to have been transfused. A review of the antibodies detected in this group revealed that anti-K was as frequently encountered as anti-D. Anti-K is considered more likely to cause a haemolytic transfusion reaction than anti-D, therefore it might be a safer practice to provide males with O Rh(D)Positive K negative blood than to provide the traditional response of O Rh(D)Negative when uncrossmatched blood is required. The practice of transfusing male patients with O Rh(D)Positive blood has conserved significant quantities of scarce O Rh(D) Negative blood and has been without incident

A review of the frequency of detection of anti-Rh(D) antibodies in antenatal patients at RNSH: correlation of variation with availability of anti-Rh(D) immunoglobulin and guidelines for use

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Since 1941, when Levine first described anti-Rh agglutinins in a case of haemolytic disease of the newborn, the role of anti-Rh(D) antibodies as the cause of haemolytic disease of the foetus and newborn has been researched and discussed extensively. In 1969 with the introduction of anti-Rh(D) immunoglobulin the number of Rh(D) negative women who develop anti-Rh(D) antibodies as a result of fetomaternal haemorrhage has decreased dramatically. Prior to anti-Rh(D) prophylaxis it was estimated as a result of pregnancy-related events that one in six (17%) Rh(D) negative women would develop anti-Rh (D) antibodies. The introduction of administration of anti-Rh(D) immunoglobulin to prevent immunisation resulted in a reduction in the frequency of pregnancy related alloimmunisation to 1.5%. In recent years, anti-Rh(D) immunoglobulin has become a limited resource and so changes in the guidelines for the administration of this product have been implemented to optimise use of the finite supply. When there was a shortfall of supply in Australia Rhogam was imported from the USA to maintain supply. The aim of this study was to review the rate of detection of anti-Rh(D) antibody in antenatal and postnatal patients at Royal North Shore Hospital. In the last six months at RNSH, for six patients anti-Rh(D) antibodies have been detected in antenatal antibody screens. In the same period last year, antibodies were detected in one patient. The data from recent years is being evaluated and correlated with changes in the guidelines for use and availability of anti-Rh(D) immunoglobulin at the time. In light of the recent release of the minidose of Rh(D) immunoglobulin and modifications in the guidelines in relation to use of anti-Rh(D) immunoglobulin, it will be interesting to monitor whether there are changes in the rate of pregnancy-related Rh(D) alloimmunisation.

Current Status Of Transfusion Adverse Reaction Reporting In Australia [A Report From The ARCBS Haemovigilance Working Party]

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Objectives: The Australian Red Cross Blood Service (ARCBS) Haemovigilance Working Party performed a survey of participants in the Royal College of Pathologists of Australasia Transfusion Serology QAP to determine the status of adverse reaction reporting to transfusion in Australia

Methodology: A written survey was mailed to QAP participants regarding the type of reporting systems in place, the infrastructure in place to support it , the number of serious reactions that occurred in the last year. Both public and private Institutions were included in the survey.

Results: Of 300 surveys, 120 responses were received. 23 serious reactions were reported including 2 ABO incompatibility and 8 non-ABO incompatibility. There were 4 TRALI, 4 anaphylaxis, 2 bacterial contamination, 1 Graft vs Host disease, 1 aseptic meningitis and 1 hypotension. Only two institutions reported having dedicated transfusion surveillance staff although most expressed an interest in being involved in an ongoing system.

Conclusion: There is a spectrum of reporting systems currently in place within Australasian end users. Awareness of the need for haemovigilance and interest in a national standardised reporting system for adverse reactions is high amongst personnel working in transfusion laboratories. However, dedicated staff performing transfusion surveillance are rare. Serious incidents such as ABO and non-ABO incompatibility do occur in Australasia indicating that one of the challenges of a National Haemovigilance System is continuing education in the broader aspects of clinical transfusion practice.

The ABMDR Eleven Years On

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Unrelated registries were established predominantly in the 1980s after results of the first major series of unrelated transplants became available. The Australian Bone Marrow Donor Registry (ABMDR) commenced tissue typing voluntary unrelated donors in December 1990. The aim was to recruit 100,000 donors in ten years. Targets were set each year and surpassed. Eleven years on by September 2001, 157,000 donors were recruited, searches were undertaken on behalf of both Australian and international patients and transplants had occurred as a result. Transplant and Tissue typing technology has changed, with very little change in allocated funding so where should the Registry go now? What are the patients rights and needs? What are the donors rights and demands? The ABMDR is no longer concentrating on just increasing the numbers. To meet the needs of Australian patients, maximising the chance of identifying a donor by recruiting the most appropriate donors while considering issues such as ethnic diversity and age is necessary. Maximising the potential for donors already on the registry by increasing the numbers that are HLA-DR tissue typed is necessary. This should help to identify donors faster. Better communication by increasing electronic communication with doctors and other international registries is also necessary. In May 2000, the Commonwealth Minister for Health and Aged Care announced funding for a National Cord Blood Collection Network. Over a four year period 22,000 cord blood units will be collected, processed, tissue typed and stored ready for transplantation. The Commonwealth plans to contract with the ABMDR to oversee the management of the funding for this development period. Availability of 22,000 cord blood units and the possibility of acceptable mismatched cord blood units will provide more patients an opportunity for treatment by transplantation. For a number of years, the Commonwealth Department of Health (now Health and Aged Care) has provided funding for searches carried out in international registries on behalf of Australian patients. $5000 is provided for every patient that meets the criteria. The cost of procuring the marrow and transport to Australia is also paid for by the Commonwealth. This ensures that all patients have the opportunity to search internationally if no donors are available in Australia. Australians continue to volunteer their stem cells for transplantation and the ABMDR continue to facilitate this not only within the Australian registry but through continued cooperation with international registries.

How We Search Smart

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Patients requiring a stem cell transplant for whom no related donor is available rely on unrelated donor registries such as the ABMDR to provide a matched donor. This can be a lengthy and costly process. Some of the factors that delay identification of a suitable donor are the hit rate of identical donors on registries, the accuracy of the donor typing results and donor availability. To maximise donor identification, we have introduced a smart searching process that utilises our knowledge of HLA genetics to target donors who are more likely to be a match.

The HLA system has evolved extensively over the 30+ years since it was first described. HLA typing was based on a variety of serological techniques and manual analysis. More recently molecular methods and sequence based typing have empirically defined the serological differences between the HLA alleles. Many previous results have been updated as a consequence of molecular data.

However, all unrelated registries are snapshots in HLA history and consequently some of the donor data is not accurate in the 21st century. As patient results are current it is important to know how an antigen and allele were previously detected when searching for donors based on historical typing data. We are able to use this information in searching and frequently find matched donors from seemingly mismatched hits. This process is effective for finding a donor, which is a significant advantage to patients and the clinicians who manage their treatment.

ABMDR: the patient

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Abstract not available at time of printing

First, find your TRALI in Australia Lookback

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Introduction: The TRALI discovery process begins with an acute, traumatic event. Recognition of this as a possible TRALI, and the reporting of this to the Australian Red Cross Blood Service, is dependent on hospital staff awareness of the significance of the combination of patient symptoms. The challenge for blood/blood product users in Australia is in the development of education programs to increase this awareness, the establishment of guidelines for dealing with suspected transfusion reactions and the introduction of a clear, accepted, reporting format. The challenge for the Blood Service is to further forge and maintain information exchange with end-users to assist with initiatives in this area.

Procedure: Blood Service medical staff are contacted with details of patient symptoms and treatment given and a decision is made as to whether a TRALI is suspected. Where this is so, copies of the relevant clinical notes, transfusion record, remnants of the transfused packs and patient blood samples are requested. Untransfused, fresh products made from implicated donations are recalled. This interim, conservative measure removes any small risk associated with these products triggering a similar event in another patient. Recalled products also provide the investigating scientist with donation samples should the units transfused to the affected patient have been inadvertently discarded. A recall of implicated donors for blood sample provision occurs if required. Donors are advised of the reason for the recall, the testing to be completed and implications for themselves as donors should white cell antibodies be detected. When all donation/donor samples are available, a 2nd patient blood sample is usually required for retrospective compatibility testing. Donors incompatible with patient samples, and all donors with granulocyte antibodies detected, are permanently deferred.

Conclusion: In cases where patient and donation/donor samples are incompatible, the initial, suspected patient diagnosis is strongly supported and a TRALI has been found . Retention of the transfused packs is paramount. Time taken to complete the investigation is significantly reduced by the availability of remnants of transfused packs or, alternatively, associated untransfused recalled products if the latter are available. The investigation process reflects an ARCBS commitment to assisting in the resolution of adverse, possibly transfusion related, patient responses, the key to resolution being the early recognition of the significance of patient symptoms by hospital staff.

TRALI a guide to definition and diagnosis

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Until recently pulmonary consequences of transfusion were thought to be rare and inconsequential. It is now appreciated that acute lung injury due to transfusion, TRALI, is one of the most important causes of morbidity and mortality. The syndrome is characterized by acute respiratory distress, hypoxemia and acute pulmonary edema within 2 hours of a plasma-containing transfusion. Fever and hypotension are frequently observed. In 80% of cases the physiologic and radiologic stigmata have improve. There are no long-term sequelae. The incidence is unknown, but it is probably under diagnosed.

However, in 6 12% of cases, TRALI leads to death. All plasma-containing blood components have been implicated. In rare cases, plasma derivatives have been involved. The pathophysiology is most likely linked to the passive transfer of HLA class I or Class II or granulocyte antibodies from the implicated blood component. In about 50% of cases, the antibody corresponds to the phenotype of the recipient. These antibodies activate complement which leads to microaggregation of granulocytes in the pulmonary vasculature. Activated granulocytes release superoxide radicals which damage the underlying capillary endothelium, resulting in pulmonary edema. Other mechanisms may also contribute. There is no recognized profile of the at risk recipient. Conclusion: TRALI is a frequent cause of severe morbidity and mortality.

Cytokines: How They Work

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Cytokines are key regulators of all immune and inflammatory responses. An understanding of how their production and activities are controlled in health and disease therefore has the potential to suggest both new diagnostic tools and new approaches to therapy. This presentation will give an overview of the major cytokines known to be important in the regulation of the immune response, their synthesis, their interaction with cellular receptors, and some of the physiological mechanisms and pharmaceutical interventions that control their activities. Particular reference will be made to the induction and functions of cytokines that determine the class of an immune response through their actions on T helper cell development.

Human platelet alloantigens

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Antibody formation against alloantigens of the human platelet membrane is responsible for clinical syndromes and transfusion related conditions as neonatal alloimmune thrombcytopenia (NAIT), post-transfusion purpura (PTP), platelet transfusion refractoriness (PTR) and passive alloimmune thrombocytopenia. Moreover, rare cases of alloimmune reactions involving platelets have been observed after transplantation of hematopoietic stem cells. Among alloantigens of the platelet membrane shared with other cells (type I alloantigens) are the glycoconjugates of the ABO system and class I human leukocyte antigen (HLA) antigens. Antibodies against these structures are responsible for PTR and for febrile nonhemolytic transfusion reations. Antibodies against type II antigens (formerly termed platelet specific antigens) have been observed in NAIT, PTP and passive alloimmune thrombocytopenia.

ABH antigens have been identified on intrinsic platelet membrane glycoproteins. Moreover, it is now clear that HLA class I antigens are integral part of the platelet membrane. Quantity of both HLA and ABH-antigen expression on the platelet membrane varies considerably. Single point mutations account for almost all platelet specific alloantigens, but most antigenic determinants seem to depend upon glycoprotein conformation: generally, platelet specific alloantibodies fail to recognize synthetic peptides encompassing the polymorphic residues.

Restriction fragment polymorphism analysis and allele-specific PCR have been implemented for genotyping of platelet alloantigens in many laboratories. Antigen specific assays using monoclonal antibodies (MAIPA, immunobead assay) became de facto standard for diagnosis of platelet antibodies in serum / plasma samples. It can be expected that innovative techniques as human alloantibody fragments produced by phage display technique and the production of recombinant antigens will allow rapid and reliable phenotyping and antibody detection in the future.

Keywords: Human Platelet Alloantigens, Structure, Alloimmune Thrombocytopenia, Diagnostics

Introduction

Antibodies against platelet alloantigens play an important role in immune-mediated platelet disorders. There are essentially three clinical conditions caused by platelet alloantibodies: Neonatal alloimmune thrombocytopenia (NAIT), posttransfusion purpura (PTP) and platelet transfusion refractoriness (PTR) (1)

In the past two decades considerable progress has been made in the characterization of platelet alloantigens. The use of serological antigen capture assays such as the monoclonal antibody immobilization of platelet antigens (2) and immunochemical methods led to rapid increase in the number of newly recognized platelet alloantigens. The introduction of PCR technology for amplification of platelet specific mRNA (3) made the characterization of platelet alloantigens possible at the molecular level (4). Based on the molecular genetic basis of platelet alloantigens new techniques for typing of platelet alloantigens and antibody detection could be developed.

Recently, human platelet alloantigens have received widespread attention beyond the field of platelet immunology. Several studies indicated that polymorphisms underlying platelet alloantigens might represent as genetic risk factors for coronary thrombosis.

In this chapter, a comprehensive review of studies, which has augmented our knowledge regarding the role of platelet alloantigens in immune-mediated platelet disorders and thrombotic disease will be given.

Platelet alloantigens / antibodies

Platelet alloantigens are defined by alloantibodies directed against genetically determined molecular variations of proteins or carbohydrates on the platelet membrane. The alloantibodies are elicited in normal individuals upon exposure to the alloantigen usually during pregnancy, blood transfusion, or rarely, by bone marrow transplantation. These alloantibodies bind to the the target platelet alloantigens, resulting in immune mediated platelet phagocytosis.

There are two different types of clinically relevant platelet alloantigens. Type I comprises common alloantigens that platelets share with other blood cells and tissues. Among these the glycoconjugates of the blood group ABH system and the highly polymorphic HLA class I molecule should be mentioned. Type II alloantigens are more or less specific to platelets and are conventionally called platelet-specific alloantigens. It is well documented that platelet specific antibodies against type II alloantigens play a role in the pathomechanism of NAIT, PTP and PTR. In contrast, clinical impact of platelet antibodies against type I alloantigens seem to be restricted to PTR.

ABH blood group antigens

Although earlier studies suggested that ABH antigens on platelets are derived by passive transfer of glycolipids from plasma (5). It is now accepted that the greater part of platelet ABH antigens is located on intrinsic platelet membrane glycoproteins. These include GPIb, GPIIa, GPIIb, GPIV, GPV, platelet endothelial cell adhesion molecule PECAM-1 and the PI-linked CD109 (6-12).

It is generally assumed that the expression of ABH antigens on platelets is too low that isoagglutins anti-A, anti-B might affect the survival of incompatible platelets. However, a number of reports have described instances in which satisfactory posttransfusion increment was only observed when ABH compatible platelets were transfused (13, 14). Recently, high amount of A or B antigens were found in 7% of A- and B-positive blood donors (high expresser) (9). Further studies demonstrated that various platelet glycoproteins, especially GPIIb and PECAM-1, from high expresser bear considerable amounts of A antigen (15). These findings indicate that heterogenous expression of ABH antigens on platelet glycoproteins of different individuals may provide an explanation for the variable platelet transfusion increment with respect to ABH incompatibility.

Human leukocyte antigens

The expression of HLA antigens on platelets can vary substantially and is influenced by gene dosage and other as yet uncharacterized genetic factors. For example, the content of HLA-B12 on platelets from different individuals varies approximately 35-fold, whereas their amount on lymphocytes is relatively constant (16). On platelets, the expression of HLA-A and -B antigens is higher than that of HLA-C antigens (17, 18). Earlier in vitro findings suggested that the presence of HLA-A,-B, -C antigens on platelets are primarily due to adsorption of HLA antigens from plasma (19). However, in vivo studies showed that transfusion of HLA-A2 negative platelets into a HLA-A2 positive recipient did not convert the HLA phenotype of donor platelets (20). Furthermore, we could demonstrate that platelets contain

specific mRNA encoding for HLA class I antigens and have the capability to synthesize the HLA membrane protein (21). These observations strongly indicated that HLA class I antigens is an intrinsic component of platelet membrane. In lymphocytes, the presence of alternatively spliced mRNA with deleted transmembrane domain resulting in secretion of soluble HLA class I antigens was documented (22). In contrast, this alternatively spliced transcript could not be detected in platelets suggesting that platelets are unable to synthesize soluble HLA antigens via an alternative splicing process (23).

Platelet-specific alloantigens

Many platelet alloantigens, previously considered as platelet-specific have been found on other cells and tissues as well. Most of these antigens are members of cell adhesion receptor, integrins, molecules known to be involved in cell-matrix or cell-cell interactions. Platelet alloantigens residing on the integrin β_3 (GPIIIa) subunit have been detected on endothelial cells, smooth muscle cells, and fibroblasts (24). Antigens associated with the α_2 integrin (GPIa) subunit have been found on activated T lymphocytes and endothelial cells (25; 26). In contrast, alloantigens localized on the α_{IIb} integrin subunit (27) and on the GPIb subunits (members of the family of leucin-rich glycoprotein) appear to be specific for the megakaryocyte/ platelet lineage.

Nomenclature and molecular genetics of platelet-specific alloantigens

Historically, platelet-specific alloantigens have been assigned designations based on patiens'names from which the alloantisera were obtained. In the course of time this nomenclature became a problem since multiple names existed for the identical antigens due to independently discovered alloantisera (e.g Zw and Pl; see table 1). To overcome this problem a new nomenclature, termed HPA (Human Platelet Alloantigen) has been proposed (28) and is now widely accepted. The different antigen systems are numbered chronologically in order of the date of their description. The high-frequency allele of a system is designated with the letter a and its antithetical low-frequency allele with the letter b (46, 47; see table 2). Six diallelic alloantigen systems (HPA-1-5 and Gov) and a number of low-frequency antigens (HPA-6bW, cf. Table 1) have been described. Currently, six platelet membrane glycoproteins GPIα, GPIbβ, GPIbb, GPIIb, GPIIIa and GPI-linked CD109 have been identified as carriers of platelet alloantigenic determinants. All six GPs are encoded by five different genes (48). Studies of a number of groups demonstrated that all platelet alloantigenic determinants characterized thus far were formed by single amino acid substitutions induced in case by a point mutation of the respective gene. The new, rare platelet alloantigen Oe^a was formed by one amino acid deletion (dLys611) of platelet GPIIIa (42).

In the HPA nomenclature, HPA-1,-2,-3,-4 and -5 were designated as separate diallelic alloantigen systems. From the prespective of molecular genetics, this nomenclature is problematic, since each new base exchange does not constitute a new diallelic alloantigen system, but rather define a single allele that expresses a single new epitope (49).

In attempt to unify the terminolgy of platelet alloantigens, a gene-based nomenclature in according to human gene nomenclature (50) has been proposed. The allelic variants of the genes encoding for human platelet alloantigenic determinants were designated as shown in table 2. However, this nomenclature does not cover the fact that a same antigen can bear different epitopes. For example, GP3A*01 allele which encodes GPIIIa*01 isoform, carry the clinically distinct HPA-1a and HPA-4a epitopes and probably many other antigenic determinants (HPA-6a...).

Structural features of platelet-specific alloantigenic determinants

Although single point mutations are responsible for the formation of platelet-specific alloantigens, the actual antibody combining sites seem to be critically dependent on the three-dimensional structure of the glycoprotein and also on the contribution of carbohydrate residues.

In the last years several laboratories have attempted to produce synthetic or recombinant peptides that mimic platelet alloantigenic determinants. All attempts to construct PI^{A1} epitopes using short linear or cyclic peptides (13-mer) straddling the Leu_{33}Pro dimorphism were unsuccessful (51, 52). Small recombinant allelic GPIIIa (66 kDa) seem to mimic PI^A epitopes (53, 54), however, some PI^{A1} alloantibodies failed to recognize this construct. Further studies demonstrated that long-range disulfide bond (Cys_5-Cys_{435}) is necessary for the structural integrity of the PI^A epitope (55, 56). Recent findings demonstrated that the actual binding site of PI^{A1} alloantibodies could be heterogenous. Some PI^{A1} antibodies bind the amino-terminal domain of GPIIIa independently from Cys_5-Cys_{435} bound and others recognize combinatorial epitopes which require this long-range disulfide bridge (57, 58).

In contrast to PI^A epitopes (51), several studies have shown that carbohydrate residues contributed critically to the integrity of Bak epitopes (59, 60, 33). Djaffar et al demonstrated that O-linked rather than presence of N-linked carbohydrate moieties preserves Bak alloantigenic determinants (61). This observation is in line with the finding of Calvete et al (62), who localized the precise site of O glycosylation to GPIIb (Ser_{847}), which is only four amino acids away from the polymorphic residue 843.

Recent findings indicate that Br alloantigenic determinants not only depend on Lys_{505}Glu dimorphism but also on glycosylation of GPIa. Analysis with different glysosylation inhibitors showed that high mannose oligosaccharides contributes critically to the formation of Br epitopes (63).

Molecular immunology of the platelet-specific alloimmune response

While the humoral response to platelet alloantigens is now well defined, the cellular basis of alloantigen recognition, processing and presentation is less clear. The immune response to the HPA-1a antigen has been shown to be strongly associated with HLA-DRB3*0101 (64) indicating the role of T cells. Recent molecular analysis showed that GPIIIa peptides containing Leu_{33} that control B cell epitopes can also stimulate T cells from a HPA-1b (Pro_{33}) homozygous mother with an affected NAIT child (65). In contrast to HPA-1a, the immune response to the HPA-1b antigen has not been found to be associated with any HLA phenotype (66). From these observations it can be concluded that presentation of GPIIIa peptides containing the HPA-1a amino acid (Leu_{33}) but not of peptides containing the HPA-1b amino acid (Pro_{33}) is facilitated by HLA-DRB3*0101 molecules. Furthermore, Wu et al (67) could demonstrate that Leu_{33} peptide bound to the HLA-DRB3*0101 molecule, whereas the Pro_{33} peptide did not. This unidirectional alloantibody response could explain the fact that HPA-1b immunization occurs rarely in comparison to HPA-1a.

Further studies indicated that susceptibility to alloimmunization to the HPA-1a antigen was also associated with polymorphism of TAP1 transporter gene (68). In the case of HPA-5b, there appears to be an association between responsiveness and the HLA DR-6 allele and TAP2 gene (69, 70).

Typing for platelet alloantigens

Genotyping

Based on the molecular genetic background of platelet alloantigens various genotyping techniques could be developed (71), which facilitated platelet alloantigen typing. To date restriction fragment length polymorphism method and allele-specific PCR are in widespread use. The development of simple rapid automated inexpensive methods may make genotyping for large number of individuals possible, as has been proposed for the screening of pregnant women for antigens of the HPA-1 system (72). To evaluate the techniques and for quality control purposes the implementation of reference DNA are necessary (73). Lymphoblastoid cell lines from typed donors have been shown as useful source for reference DNA (74, 75).

Despite its tremendous advantages, DNA-based typing techniques are unlikely to completely replace serological phenotyping in the near future. Silent HPA-1b and HPA-3a alleles leading to discordant results between genotyping and phenotyping have been reported in carriers of Glanzmann's thrombasthenia patients (76-78).

Phenotyping

Until recently phenotyping for platelet alloantigens is dependent on the availability of human sera containing platelet specific alloantibodies. Most of the sera, however, are impaired by the presence of antibodies against HLA class I antigens.

To encounter this problem Kiefel et al (2) developed glycoprotein specific immunoassay, MAIPA (monoclonal antibody immobilized platelet antigens). This technique is sensitive, realible and is one of the the standard techniques for serological phenotyping of platelets and other cells (79).

In the last years, specific human alloantibody fragments could be produced by phage display technology (80, 81). Such antibody fragments can replace the use of human alloantisera for phenotyping of platelet antigens and may be useful as therapeutic reagents.

Clinical significance of platelet alloantigens

Immunologic disorders

The most common platelet reactive antibodies in transfused patients recognize epitopes on HLA class I molecules. HLA class I antibodies frequently are responsible for febrile nonhemolytic transfusion reactions and for many cases of immunologically mediated PTR.

NAIT is usually induced by alloantibodies against platelet specific alloantigens (table 1). In the Caucasoid population, about 75% of NAIT cases are caused by HPA-1a alloantibodies. The second most frequently involved antigen is HPA-5b whereas alloimmunization against other antigens occurs rather infrequently (71). In some families alloimmunization against low-frequency antigens (less than 1 percent positive individuals) (4)

In occasional reports authors related NAIT to maternal HLA antibodies. However, results from a prospective observation suggest that in the vast majority of pregnancies, maternal HLA antibodies are not associated with thrombocytopenia in the newborn (82).

PTP is a rare transfusion reaction affecting female patients who received cellular blood products. Typically, platelet counts fall 6-10 days after a tranfusion, and severe hemorrhage may occur. With one exception known so far, high-titered alloantibodies against the GPIIb/IIIa-complex are identified in the patient's serum. The antibody is primarily directed against platelets in the transfused blood product. Although the patients' autologous platelets lack the corresponding antigen, the antibody could be eluted from the autologous platelets. This observation probably explains the pathophysiologic mechanism underlying PTP. In addition, platelet specific alloantibodies in blood donors were identified as cause of passive alloimmune thrombocytopenia, an acute transfusion reaction resulting from transfer of donor alloantibodies with blood products containing plasma. Such cases have been documented with anti-HPA-1a (for review: 71).

Platelet alloantibodies incidentally have been observed to induce immune thrombocytopenia in rare cases after transplantation of allogeneic hematopoietic stem cells. In the patient described by Taaning et al., anti-HPA-2b probably was produced by immunocompetent lymphocytes from the donor marrow (83). This condition, sometimes referred to as passenger lymphocyte syndrome has also been described for red cell alloantibodies. In a patient observed by Panzer et al., thrombocytopenia occurred in a patient with CML who received bone marrow from his HLA-matched sister. Prolonged thrombocytopenia resulted from anti-HPA-1a originating from the recipient s B-lymphocytes which were directed against platelets from the donor marrow (84).

As many glycoproteins of the platelet membrane are expressed on other blood cells and endothelial cells it was anticipated that genetic polymorphisms of such structures play a role as minor histocompatibility antigens in allogeneic bone marrow transplantation or stem cell transfer. Recently, Juji et al (85) found in patients receiving unrelated bone marrow transplantation an association between HPA-5 mismatch constellations with a reduced disease-free survival rate in bone marrow transplantation, but not mismatches in the HPA-1 to 4 systems. However, in these patients HPA-5-mismatch was not correlated with the incidence of graft-versus-host disease.

Thrombotic disorders

Since platelets play a key role in the development of acute coronary disease and cerebrovascular diseases, increasing interest has been focussed on the impact of polymorphic platelet glycoprotein structures (for review: 86). The first report on the association of HPA-1b allelic isoform of the GPIIIa (87) has induced a series of further studies. The impact of HPA-1b allele as genetic risk factor of ischemic vascular disease has been given credence by some, but not all, subsequent studies. Despite the controversy surrounding these clinical correlations, current investigations indicate that the HPA-1 phenotype has an effect on platelet function. The HPA-1b phenotype seems to confer a lower threshold for agonist-induced platelet responses and alters GPIIb/IIIa-mediated functions, such as adhesion, spreading and clot retraction. In contrast, large studies have not found an association between the HPA-3 polymorphism and coronary artery disease, myocardial infarction, or post-stent thrombosis or stenosis (88, 89). In a large cohort an association of HPA-5 with coronary artery disease in well defined patients group was observed (90). Gonzalez-Conejero et al (91) found an relationsship between HPA-2 with increased risk of coronary heart disease and cerebral vascular disease.

Conclusions

The past decades have witnessed important advances in our understanding of human platelet alloantigens. With the availability of well-defined monoclonal antibodies against platelet glycoproteins, antigen specific assays could be developed and became state of the art in many laboratories. By the use of this techniques a remarkable new series of human platelet antigens was discovered. With the introduction of PCR technology for amplification of residual platelet mRNA polymorphisms underlying platelet alloantigens could be elucidated. By the introduction of phage display technology specific human platelet alloantibodies were recently produced. These discoveries made large-scale platelet alloantigen genotyping and phenotyping available to general immunohematology laboratory. Although the molecular basis underlying platelet alloantigens is now well defined, the actual binding sites of platelet alloantibodies are still poorly characterized. Further characterization of epitopes recognized by these antibodies will aid the development of new diagnostic and therapeutic approaches for the care and management of immune mediated thrombocytopenia.

The role of platelet glycoprotein polymorphism as genetic risk factor for arterial thrombosis is a new area in this field that must be carefully evaluated. As in the case of many other previously proposed genetic factors controversies and seemingly contradictory findings abound. Well-designed, large, prospective, genetic and epidemiologic studies are needed to clarify the impact of these platelet polymorphisms. Studies of the functional relevance related to these polymorphisms should be conducted to provide biological plausibility for the clinical findings. Further interest and development in this area may give us the real oppurtunity to define adequate treatment strategies for the prevention of thrombotic disease.

Acknowledgment

This review is dedicated to Christian Mueller-Eckhardt, who introduced us into this fascinating field of platelet immunobiology.

Antigen	Synonym	Glycoprotein Location	Nucleotide Substitution	Aminoacid Substitution	Ref.
HPA-1a HPA-1b	Zw^a, PI^{A1} Zw^b, PI^{A2}	GPIIIa	T_{196} C_{196}	Leu_{33} Pro_{33}	(29)
HPA-2a HPA-2b	Ko^b, Ko^a, Sib^a	GPIbα	C_{524} T_{524}	Thr_{145} Met_{145}	(30)
HPA-3a HPA-3b	Bak^a, Lek^a, Bak^b	GPIIb	T_{2622} G_{2622}	Ile_{843} Ser_{843}	(31)
HPA-4a HPA-4b	Yuk^b, Pen^aYuk^a, Pen^b	GPIIIa	G_{526} A_{526}	Arg_{143} Gln_{143}	(32)
HPA-5a HPA-5b	Br^b, Zav^b Br^a, Zav^a, Hc^a	GPIa	G_{1648} A_{1648}	Glu_{505} Lys_{505}	(33)
HPA-6bW	Ca^a, Tu^a	GPIIIa	A_{1564} G_{1564}	Gln_{489} Arg_{489}	(34)
HPA-7bW	Mo^a	GPIIIa	G_{1317} C_{1317}	Ala_{407} Pro_{407}	(35)
HPA-8bW	Sr^a	GPIIIa	T_{2004} C_{2004}	Cys_{636} Arg_{636}	(36)
HPA-9bW	Max^a	GPIIb	A_{2603} G_{2603}	Met_{837} Val_{837}	(37)
HPA-10bW	La^a	GPIIIa	A_{281} G_{281}	Gln_{62} Arg_{62}	(38)
HPA-11bW	Gro^a	GPIIIa	A_{1996} G_{1996}	His_{633} Arg_{633}	(39)
HPA-12bW	Iy^a	GPIbβ	A_{141} G_{141}	Glu_{15} Gly_{15}	(40)
HPA-13bW	Sit^a	GPIa	T_{2531} C_{2531}	Met_{799} Thr_{799}	(41)
HPA-	Oe^a	GPIIIa			(42)
HPA-	Va^a	GPIIIa			(43)
HPA-	Pe^a	GPIbα			(44)
HPA-	$Gov^{a/b}$	CD109			(45)

Table 1: Moleculargenetic of Human Platelet Alloantigens

Kell system antigens an update

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The Kell Blood Group system currently comprises 23 antigens which are located on CD238, a red cell transmembrane single pass (type II) glycoprotein of apparent Mr 93000, a metalloendopeptidase. There are 5 sets of antigens with allelic relationships: K/k, $Kp^a/Kp^b/Kp^c$, Js^a/Js^b, K11/K17(Wk^a), K14/K24; an additional six high incidence antigens: K12, K13, K18, K19, K22, TOU and three low incidence: Ul^a, K23 and VLAN. All are governed by the KEL locus and/or expressed on the Kell glycoprotein.

Normal expression of Kell antigens is dependent on the presence of the XK protein, controlled by an X-linked gene, which carries the Kx antigen. Absence of XK gives rise to the McLeod phenotype, which is characterised by weak Kell antigens. Although primarily expressed in erythroid cells, Kell and XK are present in other tissues. The gene controlling production of Kell antigens has a chromosomal location 7q33 and is organised into 19 exons. The K/k polymorphism results from a T698C transition in exon 6 of the KEL gene giving rise to an amino acid (aa) substitution Thr193Met. This aa change affects glycosylation of the molecule.The molecular basis of other Kell antigens are also due to single aa changes. Cells with the Ko phenotype lack all Kell antigens; the molecular basis for Ko is heterogeneous.

All Kell antibodies must be considered clinically significant. Anti-K can cause severe HDN and the pathogenesis of is thought to be different from that due to anti-D.

Fetal anaemia due to anti-K is thought to result predominantly from a suppression of erythropoiesis.

Improved provision of blood for emergency use by a Retrieval Service

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Introduction: Medical retrieval services provide resuscitation and retrieval for the critically ill and injured. This may be from accident scenes or from rural hospitals. For the treatment of haemorrhagic shock, red cell transfusion is often required. During year 2000, the Royal Adelaide Hospital Retrieval Service transported blood on 38% of retrievals and transfused 117 units of blood. The provision of blood for retrievals, including the safe transport of blood in all weather, and the ability to use transported blood once returned has been poorly reported in the scientific literature, but is of life threatening importance.

Aim: To improve blood provision for the Royal Adelaide Hospital Retrieval Service by:

providing blood more rapidly

implementing a standard work procedure to help ensure appropriate blood use, and

developing a user-friendly blood shipper that will maintain blood between 2-10 C during transport. This will ensure that the blood remains safe for transfusion during the retrieval, or if the blood is returned unused, for future use.

Results: To meet the aims, a blood fridge was installed in the Intensive Care Unit to ensure rapid access for the Retrieval Service upon urgent activation. An emergency blood supply is now available without having to source it via the Transfusion Medicine Unit. A standard work procedure was introduced that provides instructions for staff members who require access to retrieval blood . It is based on the standard issue of six units of group O RhD negative blood. In association with a commercial manufacturer, a user-friendly, soft-pack blood shipper was developed to maintain blood within the acceptable temperature range for over 15 hours during transport, and validated for climatic temperatures over the range 8-35 C.

Conclusion: Provision of blood for use by the Retrieval Service is now quicker, uses a standardised approach, and blood is transported using a validated blood shipper. These factors improve transfusion practice.

Leucodepletion The New Zealand Approach

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In November 1999 the Ministry of Health announced the requirement for all blood components manufactured in New Zealand to be leucodepleted. The justification for universal leucodepletion was primarily risk reduction of theoretical vCJD transmission via blood transfusion. The target date for universal leucodepletion was set at 30th June 2001.

The New Zealand Blood Service decided as a service to develop systems for universal leucodepletion based on the approach used by the National Blood Service of England. A donor deferral program was also introduced.

Leucodepletion in New Zealand is based on two process streams, whole blood and top and bottom, with a primary and back up supplier. The Whole Blood stream involves filtration of whole blood prior to centrifugation to produce leucodepleted resuspended red cells and leucodpleted plasma. Three components are created in the initial processing of Top and Bottom packs, resuspended red cells, non-leucodepleted plasma and buffy coat. The resuspended red cells are then filtered and four Buffy Coats with one plasma are pooled by the train method to produce leucodepleted platelet pools. 70% of whole blood donations are collected into the whole blood stream, 30% into top and bottom. The target for platelet supply is 60% apheresis and 40% pools from whole blood buffy coats.

Monitoring of the process is done using NWA quality analyst software and Statistical Process Control (SPC) based on the aim to leucodeplete with 95% confidence that 99%of components manufactured have a white cell count less than 5x106/unit. SPC is based on 100% testing until 125 data points, or white cell counts, are obtained. From this data NWA is able to calculate a mean (cl), upper control limit (ucl) and lower control limit (lcl). Using the data and statistics produced by NWA a confidence report was then prepared to ensure the requirements of leucodepletion were met.

Validation was initially carried out at the pilot site NZBS Waikato. When systems were defined, equipment validated and processes documented approval was sought from the regulator, MedSafe, to release components for clinical use. As Phase one validation was completed implementation and Phase 2 began. From then on, 5 components per day, per process stream are tested for white cell contamination to ensure the process stays in control ie) within the pre determined control limits.

This presentation will focus on the principals and methods used to implement and validate the process.

Measurement of Platelet Shape Using a PACKS-4 Platelet Aggregometer

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Platelets are elliptical in their resting state, but become spherical when activated. This elliptical shape can be visualised by the amount of swirl in platelet-rich plasma and is used to assess platelet quality. A change in platelet shape can be measured as a change in light transmission through platelet-rich plasma in a platelet aggregometer. A method was developed for the PACKS-4 Platelet Aggregometer (Helena), to magnify changes in light transmission and allow an objective assessment of platelet shape.

Platelet-rich plasma (PRP) from platelet units was diluted to 300,000 platelets/ l with autologous platelet-poor plasma, and stored in air-free tubes (to maintain pH) at room temperature and 37 C for at least 30 minutes prior to testing. The PACKS-4 Aggregometer was used to measure light transmission through PRP stirred at 1000 rpm. The change in light transmission was measured, in duplicate, after activation of platelets by 10 ^{3}M ADP (adenosine diphosphate), in the presence of 0.25M ethylene glycol tetra-acetic acid (EGTA) to block subsequent aggregation.

Experiments determined which platelet concentrations were most useful for calibration of the aggregometer. Platelets from the test unit were used to avoid any differences due to the colour of the plasma. Platelet concentrations of 225,000 platelets/ l (for the 100% absorbance setting), and 375,000 platelets/ l (for the 0% absorbance setting) provided satisfactory signal amplification such that the graph obtained for the test sample (300 000 platelets/ l) was at approximately 75% of the absorbance scale.

The change in light transmission after addition of ADP was recorded by the aggregometer, and measured in millimetres on the printout. Measurements were made after incubation of PRP at room temperature and following two hours incubation at 37 C. A decreasing response to ADP with increasing age of the test unit was observed at both room temperature and at 37 C. This indicated that platelets at five days post-collection were less responsive to ADP and more spherical than platelets tested at one day post-collection.

The in vitro platelet function test which most closely correlates with platelet survival in vivo is the discoid shape of platelets and their ability to regain this shape when incubated under more optimal conditions (ie 37 C). This method can be adapted for any aggregometer which uses light transmission and provides an objective assessment of different platelet manufacturing processes and storage conditions.

Evaluation of an Integral RBC Leucodepletion System with a Reduced BC Volume

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Purpose: The aim of the study was to validate the leucodepletion performance of an integral RBC filter under three conditions, including the need to buffy coat (BC) deplete RBCs prior to filtration and the level of buffy coat depletion required for routine processing. This was also performed in the context of situations where the emphasis is on plasma as a production driver and not whole blood derived platelets.

Groups 1 reduced volume buffy coat with red cells filtered through R3000 Hard Housed filter (Asahi R3000)

Group 2 reduced volume buffy coat with red cells filtered through Soft Housed R3000 filter (Baxter OptiPure RC system).

Groups 3 no buffy coat removal with R3000 Hard Housed filter (Asahi R3000).

Method: Blood was collected from donors meeting all the usual ARCBS collection criteria and a reduced volume for the buffy coat was determined that did not compromise the quality of the resulting products. Whole blood donations were processed, then filtered immediately after processing at ambient temperature. RBCs and plasma where tested the day of filtration for quality. RBCs where subsequently re-tested at expiry.

Results:

Group	BC	Leucodepleted Red Cell Concentrates			Plasma
	Vol (mL)	Vol (mL)	Hb (g/unit)	WBC	Vol (mL)
1 (n=20)	33–5	257–10	50–3	$<1x10^6$ per unit	306–11
2 (n=18)	35–4	256–19	50–6	$<1x10^6$ per unit	303–17
3 (n = 6)	Not removed	No result	No result	No result	307–17 (previous study)
Optipress II Validation	59–1				298–17

Conclusion: RBC filtration using the in-line R3000 collection bag provides leucodepleted RBCs. The advantage of a reduced BC is an additional 5 8 mL of plasma from a standard volume buffy coat unit. In addition, the system is easy to use within blood banks to meet their leucodepletion needs where whole blood derived platelets are not a production driver. The study also demonstrates that the initial RBC product must be buffy coat depleted to allow the RBCs to pass through the filter, and thus provide a leucodepleted product.

Pathogen inactivation of platelets

Peyton Metzel (video presentation)

Abstract not available at time of printing

Future of natural and engineered products in Europe

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The life cycle of therapeutic proteins derived from human plasma, which started 30 to 60 years ago, has turned out to be longer than for many pharmaceuticals. Alternatives for some of these products have become available when the industrial application of recombinant techniques has created possibilities to prepare proteins from non-human sources. The increased awareness of the risk of virus transmission by plasma products following the AIDS-epidemy and the limited supply of some convential products derived from plasma (such as factor VIII concentrate) have become major elements in the competition between natural and engineered products. However, expectations implying that the need for plasma derived products will soon disappear once recombinant products are on the market, have not (yet) come true.

Although the unlimited supply of products was one of the promises of recombinant technology to cause changes from natural to engineered plasma products, in reality it proves to take much longer before the recombinant plasma products are capable to meet the clinical demands. Limited manufacturing capacity, GMP-problems during the production of recombinant proteins, the increased usage of clotting factor concentrates and the concern about inhibitor formation after recombinant products have kept the need for convential factor VIII concentrates intact. At the same time new plasma proteins (like albumin, Von Willebrand factor, protease inhibitors, activated clotting factors) for clinical application have been developed using recombiant technology. Transgenic products such as alpha-1-antitrypsin will also become available. Thereby the package of plasma products, whether convential or engineered, will offer possibilities for the treatment of a wider spectrum of diseases. To which extent and at which moment gene therapy will change the need for natural and recombant productswill depend on the outcome of safety and efficacy studies. Other developments in molecular biology, molecular life sciences and genomics are likely to offer further opportunities for optimal treatment .

Future of natural engineered products in Australia

Jerry Kanellos

Abstract not available at time of printing

How to make a new plasma-derived hyperimmune

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Historically, plasma-derived antibody-based products have been divided into generic and a smaller group of hyperimmune products that are derived from specific donors. Generic product (so called IVIG) has been used to treat a number of autoimmune and immunodeficiency diseases while specific hyperimmune-based products have been developed for Tetanus, Hepatitis A and CMV (this list is by no means exhaustive).

As part of a proof of principle study funded by the CRC-Vaccine Technology, we have been investigating the feasibility of developing a hyperimmune product for the treatment of Pneumococcal pneumoniae. At risk groups include the elderly and young infants and morbidity and mortality rates remain high in these groups.

Selection of this candidate organism (Streptococcus pneumoniae) was based on a survey of infectious disease specialists and a number of additional criteria, which included disease burden, antimicrobial resistance and the commercial availability of a vaccine.

As a first step we have screened a large number of plasmapheresis donors and using a reference serum prepared from vaccinated individuals, have obtained a seroprevalance rate of 6% in this donor population. Present work is being directed towards identifying the serotype specificity and assessing the protective effect of these antibodies using an in vitro opsonophagocytosis assay.

While the eventual decision to proceed with a Pneumococcal hyperimmune-based product will be based on a number of commercial and scientific factors, the present work highlights the value of this approach and furthermore this approach could be readily applied to any number of other pathogens.

Development of Anti-Chido following a Transfusion of Platelets

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Background: Chido (ISBT017.001) is of high incidence, but is not located on intrinsic red cell structures. It is located on the fourth component of complement (C4), which is adsorbed onto the red cell from the plasma.

Case Study: Patient KH with previously reported platelet antibodies and no history of red cell transfusion was admitted for surgery (hysterectomy, hernia repair and dental work). There was no evidence of red cell antibodies and crossmatch compatible platelets were issued and transfused uneventfully over a period of 8 days. Post-transfusion the patient s haemoglobin dropped to 73g/L and red cell units were requested. The antibody screen was positive and the serum of patient KH contained an antibody to a high incidence antigen following the multiple platelet transfusions.

Results: KH serum gave variable strength reactions with all cells, except Ch( ) and her own cells and the antibody did not react with papain treated cells. Coating of weakly positive cells with C4 from fresh normal human serum enhanced the reaction strength of the antibody, which suggested Chido or Rodgers specificity. Inhibition studies using plasma from Ch( ) and Rg( ) donors confirmed the specificity of the anti-Ch antibody. Discussion: This report describes an example of anti-Chido stimulated by multiple transfusions with crossmatch compatible platelets.

Maternal Antibodies and Haemolytic Disease of the Newborn: New Laboratory Tests of Potential Value in Clinical Management

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A study has been made of pregnant women referred to the Fetal Medicine Unit for clinical management because of the presence of maternal antibodies known to cause HDN. Thirty patients have been included in this survey, 24 of whom had antibodies to Rh(D), 2 to K(1), 3 to c and one to Fy^a. One of the aims of the project has been to quantitate antibody titres by flow cytometry, comparing these values with conventional haemagglutination titres, and where possible, with AutoAnalyser quantitation. In addition, the subclasses of specific antibody present in the maternal plasma have been identified and quantitated by flow cytometry, using mouse monoclonal antibodies to the IgG subclasses 1 4. The results of subclassing have been correlated with antibody quantitation and with clinical outcome. The antibodies in a small number of patients have been subclassed also by a new gel column test DAT IgG1/IgG3 . In the present series with an antibody predominantly IgG1, the results of AutoAnalyser quantitation, titre (by haemagglutination or flow cytometry) and clinical severity are in close agreement, whereas when the subclass is predominantly IgG3 this is not the case. The AutoAnalyser results for such antibodies are consistently low, seriously underestimating the potential risks to the fetus and emphasizing the importance of knowledge of the subclass.

Of the 24 patients with anti-D, 10 have been given between 1 and 5 intrauterine transfusions to maintain the pregnancy. Three of these patients have had two consecutive pregnancies managed in this way. Flow cytometric techniques have allowed quantitation of the maternal antibody in the fetal circulation, quantitation of the remaining fetal red cells and provided a tool for measurement of the half-life of the red cells from individual intrauterine transfusions. In 3 instances, baby heel prick samples have become available months after the birth to confirm the length of time maternal antibody may persist in the circulation of the baby.

In our experience, flow cytometric titrations more accurately reflect the potential for haemolytic disease of the newborn compared with conventional haemagglutination titres, and the ability to subclass and quantitate the IgG antibody is of particular importance.

Polyethylene glycol (PEG) enhanced adsorption to isolate alloantibodies with free autoantibodies: a two year experience of a routine blood bank laboratory

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The presence of free, warm autoantibodies in a sample for crossmatching may mask the presence of underlying alloantibodies. Eleven to forty-seven percent of these cases contain clinically significant alloantibodies. It is necessary to incorporate procedures into the routine of blood bank laboratories to identify these antibodies and to offer compatible blood for transfusion with minimum delay. Adsorption methods using PEG in a low ionic strength medium has been shown to be rapid and sensitive. In this study thirty-one samples from different patient have been tested in the two years since adopting these techniques. Fifteen (48%) of these samples contained alloantibodies. Autoadsorption, using the patient s untreated cells as the adsorbing medium, was appropriate in ten cases and four of these revealed alloantibodies (Rh, Lua and Bga). Twenty-one cases, with recent history of blood transfusion, required differential adsorption using three selected, allogeneic cells as the adsorption media. Eleven of these sera contained underlying alloantibodies (Rh, K, Jk^a, Fy^b, Fy^a, Le^a, Kp^a and Bg^a). Either a single absorption phase(2), double adsorption phases(22) or triple adsorption phases (7) were necessary. There were no adverse patient outcomes, judged clinically and serologically, to the blood selected for transfusion by this technique. The method s simplicity, speed and efficacy is suited to the routine domain. Its application allows confident selection of blood for transfusion of these problem patients.

Autoimmune anaemia associated with high incidence Kell blood group system antibody and negative direct antiglobulin test

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Case Presentation: A 28-year-old female presented with profound lethargy and SOB and was investigated for a severe normochromic, normocytic anaemia. Laboratory tests revealed haemoglobin of 51g/L with a reticulocytopenia. She had recent history of a mild upper respiratory tract infection and previous history of Sjogrens syndrome that was in remission. The blood film revealed no morphological evidence of haemolysis, the bilirubin was normal and the DAT was negative. Bone marrow biopsy showed marked erythroid hyperplasia with no dysplastic features. Pretransfusion testing was requested prior to the transfusion of red cell concentrates to correct her anaemia. She was treated with blood transfusion and prednisilone and made a full recovery.

Laboratory Findings: The patient s serum contained an antibody by indirect antiglobulin test that reacted with all red cells tested except autologous red cells, AET treated red cells, K_o and McLeod red cells. The NSW Red Cell Reference Laboratory confirmed an antibody to an unidentified high incidence Kell system antigen. Extended red cell typing showed her cells to be markedly suppressed or negative for Kell system antigens and typing as K: 1,2 (weak), 3, 4, 7, 11.

In vitro tests established the antibody s ability to suppress bone marrow erythroid progenitors in clonal culture. Normal bone marrow cells were grown in culture with the patient s serum, or with a high titre anti-D as control. The results are as follows:

	Erythroid BFU at day 14 (per well)	Average BFU (per well)
Control (no additive)	113, 128, 133	125
Control serum (20% anti-D)	153, 161, 144	153
Kell antibody (20%)	56, 48, 63	56

Conclusion: The in vitro evidence suggests that this Kell system antibody was capable of suppressing erythroid progenitors and was the cause of this patient s anaemia. She was transfused a total of 11 red cell concentrates over a 2 month period and received prednisilone as immunosuppression. Three months following the resolution of her anaemia, antibody screen and phenotyping was repeated. No red cell antibody was detected in her serum and her red cells exhibited normal expression of the common red cell phenotype: K: 1, 2, 3, 4, 6, 7.

Does red cell T activation warrant routine screening protocols and special transfusion precautions in neonates? The debate

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Worldwide, management policies for microbial enzyme induced T cryptantigen activation (TCA) differ due to debate on the clinical and laboratory significance of TCA and whether routine screening protocols with the peanut lectin Arachis hypogea and special transfusion strategies are necessary. The key issue is whether a true cause and effect relationship exists between the infusion of IgM anti-T in standard plasma-containing blood products (SP-CBP) and intravascular haemolysis of red cells with TCA. Policies include: adoption of no preventive measures; a selective testing strategy with the avoidance of SP-CBP and provision of low titre anti-T plasma-containing blood products (LTA-TP-CBP); or outright refusal to provide any blood products to patients with TCA. TCA is rare in healthy perinatal populations but occurs in up to 17% of septic surgical neonates (SSN) and in 11 34% of neonates with necrotising enterocolitis (N-NEC). In a study of paired maternal and cord blood samples we found TCA in 2.2% (10/455) of maternal and 2.8% (15/544) of cord bloods respectively. No paired samples were positive and only one infant with an omphacoele required transfusion. Over a 12 month period we screened neonates deemed to be at high-risk based on their clinical history and found TCA in 0 of 19 septic medical neonates, in 3 of 4 SSN and 4/9 N-NEC. In the latter group, three of four had fulminant NEC with bowel perforation and required surgery. In infants with TCA none of the SSN and none of three N-NEC transfused with high haematocrit paediatric packs (HHPP) or LTA-TP-CBP (kindly provided by the Australian Red Cross Blood Transfusion Service, Brisbane) developed intravascular haemolysis. One infant with NEC and TCA negative on initial testing was given SP-CBP and developed intravascular haemolysis and TCA and subsequently died. In a recent 5 year review of infants with NEC (n=59), we found those with TCA had a significantly higher likelihood of advanced disease (100% [5/5] vs 48% [26/54] $p<0.05$), bowel perforation (80% [4/5] vs % 7.4% [4/54] $p<0.001$) and surgical intervention (100% [5/5] vs 24% [13/54] $p<0.005$). None of the three infants with TCA who received HHPP, and/or LTA-TP-CBP developed haemolysis. One infant initially TCA negative, who was given SP-CBP during transport to our unit, had severe intravascular haemolysis and TCA on arrival. We have seen only one N-NEC with intravascular haemolysis who was negative for TCA. We conclude that in N-NEC, TCA identifies a group with fulminant disease and a high likelihood of bowel perforation and surgical intervention and who require strict transfusion precautions. In order to provide the full range of blood products for the transfusion emergencies that arise in N-NEC, we recommend the use of LTA-TPCBP regardless of TCA status.

Neonatal Alloimmune Thrombocytopaenia Laboratory diagnosis and transfusion

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Neonatal Alloimmune Thrombocytopaenia (NAIT) or Foeto-maternal alloimmune thrombocytopaenia (FMAIT) is the platelet equivalent of haemolytic disease of the newborn. The foetal platelets are destroyed in utero by maternal antibodies produced as a result of maternal immunisation induced by paternal human platelet antigens (HPA) inherited by the foetus.

In a recent prospective study of 24,417 pregnancies it was found that the incidence of HPA-1a antibodies occurred in 1/350 unselected pregnancies and that the incidence of neonatal thrombocytopaenia was 1 in 1,100. Intra-cranial haemorrhage (ICH), the major cause of mortality and long-term morbidity occurs in 15 20% of cases. While there is a serious risk of ICH at delivery, nearly 50% of ICH occur in utero. From these figures it would appear that FMAIT is under-diagnosed in our population.

The most common cause of FMAIT in the Caucasian population is anti-HPA-1a followed by antibodies directed to other HPA. Antibodies to Gov antigens have been more recently implicated.

As the firstborn is affected in more than 60% of cases, and as there is no antenatal screening for the condition, the requirement for compatible platelet transfusion is often urgent and unpredictable. In our experience, rapid serological and phenotyping or genotyping assays, and then the availability of platelets derived from a fully genotyped and counselled donor panel, are fundamental to the provision of appropriate transfusion support.

The primary serological investigation is performed within 2 hours with a solid phase red cell adherence assay utilising frozen panel platelets (SPRCA F) stored at 80 C. HLA-stripped panels (acidified chloroquine) are included ensuring that HLA and HPA alloantibodies are separated with initial testing. The MAIPA assay is performed as a follow-up investigation.

As anti-HPA-1a is implicated in more than 80% of cases, our strategy has been to phenotype primarily for HPA-1a with broad-scale donor screening. Donations are also phenotyped for HPA-3a and HPA-5b. Donors with desirable phenotypes are then targeted for full HPA genotyping. Now that sufficient HPA-1a negative donors have been found, rostered donations ensure that at least one HPA-1a negative platelet concentrate is always available. As the donation is fully genotyped it may also be suitable when antibodies to other HPA are implicated.

Strategies for future pregnancies include genotyping of parents and foetal amniocytes when the father is heterozygous. When intra-uterine sampling and platelet transfusion (IUT) is indicated, or the infant is premature, consideration is given to the use of plasma-reduced platelet concentrates or hyperconcentrated platelets.

Neonatal blood transfusions: policies and procedures

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Blood transfusion in neonates can be classified as acute, early, top up , post discharge and exchange and dilutional. Simple red cell transfusions are most commonly given for symptomatic infants with exaggerated anaemia of prematurity. This form of anaemia can be minimised by delayed cord clamping, restricting iatrogenic losses or erythropoietin therapy. Paediatric packs (Quad packs) are collected from selected donors and are leucocyte depleted, CMV negative, irradiated and have high Hct 0.7 0.8. Packs have an expiry date of only 10 days because of potentially high levels of potassium. Guidelines for transfusion instituted in July 1999 at Mater Mothers Hospital (MMH) are restrictive and do not include transfusion for low Hct alone. Implementation of these guidelines resulted in modest decreases in requirement for transfusion in 1999/2000 compared with 1996 1998 for infants 500 749g, 750 999g and 1000 14999g birth weight. When the median number of transfusions for MMH was benchmarked against King George V Hospital (KGV), Sydney for infants 24 20 weeks gestation there was little difference except at 24 weeks (MMH 5 transfusions, KGV 7) and more frequent transfusions at 28 30 weeks for MMH babies.

The published literature on the consequences of neonatal transfusion in terms of heart rate, apnoea/bradycardia, weight gain and subclinical tissue acidosis has been reviewed.

In summary, Queensland has a high quality blood transfusion services for neonates. Compliance with transfusion policy in 2000/2001 should be audited. Benchmarking between tertiary perinatal centres might well further modify practice.

What has vCJD cost us?

Peter Flanagan
New Zealand Blood Service

There is no evidence that vCJD has ever been transmitted by transfusion of blood or blood products. Increasing concern, supported by limited scientific data, has however resulted in the introduction of a series of precautionary measures.

During 1998 the United Kingdom introduced a package of measures to reduce the risk of vCJD transmission. Other countries began to consider what steps might need to be taken to protect their own blood supply. In September 1999 the US FDA and Health Canada jointly proposed the introduction of a donor deferral based on greater than 6 months residency in the United Kingdom between 1980 and 1996. This approach has since been introduced in a number of countries, including New Zealand and Australia.

The introduction of the UK donor deferral in New Zealand has resulted in the loss of approximately 10% of the donor base. A major recruitment campaign was undertaken to replace those donors lost as a consequence. This was successful. Retention of these new recruits has been more problematic.

The US FDA is currently considering whether precautionary measures should be extended to include restrictions based on residency in Europe. This is being considered in the context of proposals from the American Red Cross that would result in the loss of 8-9% of US donors.

The European Plasma Fractionation Association has raised concerns that the inconsistent application of precautionary measures might lead to restricted availability of fractionated blood products.

It is too early to assess the full impact that the application of the precautionary principle in relation to vCJD will have on Blood Services. It will be important to ensure that wider issues in relation to supply of fractionated products are considered as the debate on extension of precautionary measures continues.

Decreasing the risk of allogeneic blood in the 21st Century

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Despite impressive improvements in blood safety over the last 15 years, transfusions may still cause serious complications. Future improvements will likely be in the following areas: 1) donor medical history screening; 2) modification of transfusion practices; 3) decreasing allogeneic donor exposures; and 4) pathogen inactivation. The medical history may be modified with ever more restrictive blood deferral criteria and the use of computer-assisted histories. Donors have been shown to be more truthful with computers than with traditional oral medical histories. Techniques have been developed which can modify transfusion prescribing behaviors. This technique, academic detailing, results in significant (30 40% improvement) long-term changes in how red cells are transfused. Additionally, technology is available which can reduce risk to recipients by decreasing donor exposures. These technologies include 2-unit red cell apheresis in which both units are transfused to the same recipient and intraoperative red cell salvage. The ultimate technology to make blood safer may be pathogen inactivation of cellular blood components. This technology is not yet commercially available but could be introduced within 2 years. Pathogen inactivation of platelets and red blood cells involves the introduction and removal of an agent which inactivates viral and bacterial DNA.

The cost to the Australian community of blood and blood products

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Australian Red Cross Blood Service Melbourne

This paper will provide an overview of the cost to the Australian community of blood and blood products.

The cost of producing blood and plasma products needs to be assessed against the backdrop of most products being provided by blood donations from voluntary non-remunerated Australian donors. Costing data in relation to blood and plasma products produced and distributed by the Australian Red Cross Blood Service will be presented utilising a marginal costing model of plasma fractionation, and imported products will be presented separately.

The UK universal Leucodepletion strategy: The lessons learned and new developments

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The implementation of universal leucodepletion in the UK-NBS has created some new requirements, ie.: validation/standardisation of leucodepletion processes and sampling/counting methods to improve the inter-changeability of results; the implementation of an appropriate testing schedule, performance monitoring, statistical review of processes, conformance checks and stability monitoring in process; and setting up contingency plans and remedial action in the event of a process failure. A 3-monthly analysis of National data reveals continual improvements in specification targets for all types of leucodepletion processes and products. Rare but random failures are seen in some donations with minor haematological abnormalities. Blockage and atypical leucocytes are consistently seen in red cells derived from sickle cell trait, requiring a new screening procedure. From the plateletpheresis standpoint, some donor related issues were identified and remedial actions are in place, substantially reducing the frequency of leucodepletion failures. In some cases the frequency of double dose donation is related to the release of atypical WBC, with smaller size and poorer retention on filters. Overall, leucodepleted products derived from various leucoreduction processes or leucofiltration differ in terms of some specific markers, such as the levels of leucocyte subsets, microvesicles, cytokines, soluble HLA-1, release of bradykinin and complement activation. The key issue is not the number of residual leucocytes but their characteristic properties which may influence the overall clinical outcome. So far the universal versus selective leucoreduction processes , using 3-4 log reduction had little effect on abrogating FNHTR, bacterial contamination of platelets and may not eliminate the risk of HTLV transmission but bedside filtration is not recommended.

Australian leucodepletion journey and signposts

Sally Thomas
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Abstract not available at time of printing

Validation of the conditions for use of an in-house clonogenic assay control

Gail Lazzaro
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Abstract not available at time of printing

Stability of Duffy and CD47 Antigens in Stored Red Blood Cell Products

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The stability of many red cell surface antigens under storage conditions is unknown. In view of the important role the Duffy antigen plays in chemokine-scavenging, the aim of this study was to determine if Duffy antigen expression was maintained in aging erythrocytes under normal storage conditions. This study also aimed to examine the stability of CD47, a molecule recently demonstrated to be a marker of self in circulating erythrocytes. Loss of this self-recognition antigen under storage conditions may leave transfused erythrocytes unprotected from destruction by splenic macrophages thereby reducing the benefits gained by transfusion.

Whole blood was collected from 10 donors into Baxter Fenwal packs containing CPD anti-coagulant. Hard-spun red blood cell concentrates were then prepared according to standard blood bank procedures. Red cells were stored in Adsol storage solution for 42 days at 4 C with samples collected aseptically on Day 1, 14, 28 and 42. On the day of sample collection, red cells were diluted to a concentration of $2x10^6$ RBC/ml. Cells were either labelled with anti-Fy6 (Duffy) and FITC-anti-mouse Ig $F(ab)_2$ for Duffy antigen expression or anti-CD47-PE for CD47 antigen expression. The samples were analysed by flow cytometry against appropriate isotype negative controls. The flow cytometer was calibrated each time to ensure identical instrument parameters. In addition, Duffy phenotype (Fy^a and Fy^b) was also monitored throughout the storage period using rountine red cell serology procedures.

No significant change in fluorescence intensity was detected in erythrocytes labelled with Fy6 thoughout the 42 day storage period. In addition, no change in strength of Fy^a and Fy^b alloantigens were observed. However, a small but significant change in fluorescence intensity was observed in erythrocytes labelled with CD47-PE antibody indicating a loss of CD47 cell surface antigen in erythrocytes older than 14 days ($P<0.05$). Interestingly, this significant reduction in CD47 antigen expression was only observed in the Rh-positive erythrocyte subgroup with no change observed in Rh-negative cells.

Our Fy6, Fy^a and Fy^b results show that Duffy antigen expression remains stable during the 42 day storage period. This result indicates that a loss of Duffy antigen numbers does not contribute to any reduction in erythrocyte chemokine scavenging ability. In addition, our finding of a small but significant reduction in CD47 antigen expression in erythrocytes during storage is of particular interest in a transfusion setting but its significance will require further examination.

Evaluation of Haemonetics MCS+Leuco-depleted Platelet Revision C.2 Software: the Apheresis Perspective

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Introduction: To improve service delivery to donors and product quality, Haemonetics have introduced revised software for the operation of the MCS+ Leucodepleted Platelet Protocol (LDP)

This software offers:

A brief spin of the centrifuge bowl to ensure correct installation before priming the disposable
Continuous filtration of platelets during the collection
Improved air management during the procedure resulting in less air in the final product.
Enhanced bowl optics reference algorithm to avoid red cell spillage if the plasma is lipaemic.
Improved platelet elutriation using the optical density of platelet components. (Fuji-surge)
Control of the maximum platelet concentration.
The introduction of plasma to the platelet volume to increase the maximum platelet volume.
Better management of the anticoagulant reinfusion rate to enhance donor safety and comfort.
The volume of anticoagulant in each product is displayed in the final procedure statistics.

The purpose of the study was to evaluate the impact of Rev C.2 software on provision of quality services and product.

Method: 45 platelet donations were collected with Rev C.2 software on the Haemonetics MCS+. Apheresis staff evaluated the collection process with particular consideration to donor comfort, duration of procedure, installation of kits and display of procedure statistics.

Results: The following major benefits were identified when comparing the revision C.2 software to revision C.

A brief spin of the centrifuge bowl during pump loading ensured correct bowl installation therefore eliminated the risk of product loss due to bowl shear.
Continuous filtration of product eliminated the risk of product loss due to failure to install the line from the collection bag to the storage bags.
Citrate infusion rate determined by donor characteristics and plasma volume to be returned enabled an automatic return rate appropriate to the individual donor.
Final procedure data displayed the volume of anticoagulant in each product enabling the calculation of the volume of actual plasma collected.

Conclusion: Process improvement as a result of the Rev C2 software offers significant benefits to staff, donors and end users.

The Long Term Effects Of A Short Term Blood Donor Recruiting Program 18 Months On

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Background: The Geelong Blood Centre is a 7-couch whole blood collection and a 4-couch apheresis collection static blood centre. A theme-focused, short-term blood donor recruitment program, based on the potential for the Y2K Bug to interfere with critical systems and negatively impact on blood supply was undertaken from November 1999 to January 2000. From this experience, it was realised that the Geelong Blood Centre had a much greater potential for donor recruitment and donor retention. A proposal was put forward to more fully utilise the Centre. The details of the background planning and activity to realise this vision are presented.

Aim: This data aims to highlight the activity and planning that influences the collection of blood and the cost effectiveness of collection strategies.

Method: A staged increase in staff occurred to cope with the projected increase in activity. In the first two months a concentrated, strategically planned marketing effort was put in place, using tools such as e-mail and fax advertising, incentives for new donors and encouraging existing donors to recruit new donors. The program was specifically designed to have maximum effect over a short time frame. When the results of this short-term project were analysed, it was realised that Geelong Blood Centre had a much greater potential for donor recruitment and donor retention. A proposal was put forward to more fully utilise the potential of the Centre. A range of activities helped sustain the growth already started (such as focusing of resources both in donor collection personnel and marketing activity). The following approaches were utilised to maintain the momentum established by the initial program accurately understanding donor needs and expectations, advertisements with a local content, studying demographic data to target likely pockets of growth, staff involvement in recruiting, strong relationships with service clubs, community and sporting groups, strong interaction with the local media. It was hoped to increase the number of whole blood units collected by 5000 above previous operating level.

Results:

YEAR	WHOLE BLOOD	PLASMA	TOTAL	% INCREASE ON 1998 1999
1998 1999	8180	3091	11271	
1999 2000	11231	3209	14440	28.1
2000 2001	13205	3763	16968	50.5

Conclusion: This project demonstrates the powerful effects of targeted marketing in increasing donation numbers. The number of donations dropped significantly in January, coinciding with a cessation of marketing. In March, a secondary rise in collections was seen as the new donors recruited in November and December 1999 returned to donate again. This growth in donations from these targeted recruits has been sustainable and would suggest that a short-term targeted donor-recruiting program has the potential to result in long term benefits in terms of increased blood donations.

Emerging viral diseases in the Australasian region: Do they present a threat?

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Emergent viral diseases in Australia are, with a few important exceptions, not dissimilar in their identity, occurrence and patterns of incidence to those described in industrialised countries elsewhere. Most of the exceptions are either vector-borne or zoonotic viral diseases, and they fall into two patterns; known diseases which are increasing in incidence and/or geographic spread, and novel diseases which have not been recognised previously. The former include mosquito-borne virus diseases such as Japanese encephalitis (JE) virus and dengue viruses, whereas the latter are zoonotic diseases such as Hendra virus, Menangle virus, and Australian bat lyssavirus (ABL).

Of the mosquito-borne diseases, the greatest threat is undoubtedly JE virus. To date there have been five clinical cases of JE, four on one of the islands in the Torres Strait and one on the Australian mainland, and almost 60 virus isolations from mosquitoes and pigs. The isolates of JE belong to two distinct topotypes; most isolates belonging to topotype 2, which normally extends from southern Thailand through Malaysia and Indonesia; but isolates obtained in early 2000 were found to belong to topotype 1, which includes viruses from northern Thailand and Cambodia, with occasional isolates reported from Malaysia and Korea. Australia has suitable vertebrate hosts and vectors for the virus to become enzootic, and indeed potential wildlife hosts are widely available in both rural and urban settings. All four types of dengue have been introduced into the dengue-receptive areas of north-eastern Australia over the past decade, and epidemics of three types have occurred.

Of the novel zoonotic diseases, Hendra virus was the first to be recognised during an outbreak in horses in Brisbane. This was shown to be a natural virus of fruit bats, and to be widely distributed in northern and eastern Australia, as well as PNG. Hendra virus has been classified as the first member of a new genus in the family *Paramyxoviridae*. A second member of this new genus emerged in 1999 in Malaysia, Nipah virus. Studies of Hendra virus led to the discovery of ABL, a rabies-like virus, in both fruit bats and insectivorous bats. ABL belongs to antigenic group 1 of the lyssaviruses, which is the same as classical rabies, but can be differentiated on genetic grounds. The most recent virus, Menangle virus, was recognised as the cause of an increased incidence of foetal death in a commercial piggery, and as a possible cause of influenza-like illness in humans. It also appears to be a virus of fruit bats, and is a member of the *Rubulavirus* genus of the family *Paramyxoviridae*.

Finally, the importance of epizootics in wildlife is of growing concern because of their possible zoonotic potential. Thus there is a recognition of the necessity for improved infectious diseases surveillance, and of the need for public education and awareness of the possibility of transmission of diseases from wildlife to humans.

Thus, while there is considerable concern associated with these viruses and their involvement in human health, as well as their potential long-term public health implications, they do not pose any immediate threat to blood or blood products.

Hepatitis C virus and vaccine developments

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Around 80% of individuals who are infected with hepatitis C virus (HCV) develop persistent infection. As a result, it is has been estimated that there are approximately 200 million HCV carriers globally. These carriers are not only at risk of developing serious liver disease, but represent a reservoir for transmission to uninfected individuals. Although transmission of HCV occurs mainly by intravenous drug abuse in Western countries, nosocomial transmission is a major route in developing countries and is also recognised in developed countries. Consequently, because individuals at risk are often not readily identifiable or accessible, a vaccine for the general population is necessary.

Without a robust cell culture system which could be used to generate an attenuated virus strain, the development of a vaccine for HCV is challenging and consequently, it will be necessary to use recombinant DNA technology. However, the problem is exacerbated by our lack of understanding over what constitutes protective immunity against HCV infection. Although neutralising antibodies against homotypic virus can be effective, HCV circulates as a quasispecies in infected individuals, and this results in the appearance of neutralising antibody-escape mutants. A CD4+ T cell response, particularly to the virus non structural proteins, is associated with recovery from acute infection, as is a multispecific ongoing CD8 cytotoxic T lymphocyte response.

Consequently, the challenge is to generate a cellular immune response without the benefit of a live attenuated virus. Several alternative strategies have been described by various research groups, including DNA immunisation and, although some formulations have generated such a response, these experiments have been restricted as yet to mice which have been challenged with a surrogate virus viz. recombinant vaccinia virus, because the only animal model for HCV infection is the chimpanzee.

Greater Safety Assurance: why and at what cost?

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The plasma fractionation industry has now reached the point where existing measures (donor screening, plasma testing and viral inactivation/removal) already provide a high degree of safety and any further additional elimination or screening should be seen as addressing heightened public perception of safety. For most products it has become difficult to demonstrate significant benefits in further changes as these benefits can only be shown if significant risks already exist. Recent changes to regulatory guidelines have placed greater demands on screening requirements with mandatory nucleic acid amplification testing (NAT) for HCV, and in some countries HIV, to reduce the window period. In addition to HCV and HIV, manufacturers are now looking at screening for HBV and HAV and whilst these measures have a cost implication for little practical safety return in processes with efficient inactivation/removal steps, there is little impact on plasma supply or production. In contrast, industry trends of enhancing safety for the ubiquitous human parvovirus B19 (B19) by NAT screening of plasma has the potential to reduce the available production plasma by up to 1%. Substantial loss of available plasma for fractionation has occurred with the United Kingdom donor exclusion policy as a precautionary measure against the theoretical possibility of vCJD transmission. The loss of plasma, and ultimately final product, by enhanced screening can be exacerbated further by introducing inactivation steps that may have an efficacious virucidal activity but affect adversely the final yield of product. The common goal of the patient, fractionator, health care worker, regulator and government for zero risk will continue to be pursued but eventually the increased assurance of safety must be weighed against the inevitable loss of supply of product and medical need.

Albumex

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Abstract not available at time of printing

Triggers and Targets - IVIg

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The chance discovery of the immunomodulatory effects of highly purified monomeric suspensions of immunoglobulin given intravenously (1981) lead to a dramatic expansion in the therapeutic use of, and demand for, such preparations. Despite the institution of a variety of rationing and regulatory mechanisms to manage the resource, blood product services have struggled to keep up with demand for existing, let alone new indications. The ASBTs 1992 Guidelines were rapidly adopted as the Australian Standard and remained in use until the end of that decade. However, and despite the Federal Government s 1994-5 annual $8m plasma funding initiative, production and supply constraints in the latter half of the 1990s continued to elicit angst and much criticism amongst consumers. In particular, some clinicians perceived an increased and unmet need for the use of IVIg in a variety of neurological disorders. As a result the Australian Health Ministers Advisory Council Blood and Blood Products Committee recently reviewed this issue. Key recommendations of their report include:

1 That ARCBS and CSL undertake ongoing collaboration to ensure optimisation of production in relation to demand.

2 The establishment of a national policy to ensure equitable access to all.

The latter was based upon a reanalysis of the evidence for the use if IVIg and the assignment of prioritorised categories (1,2,3) based upon the principles of evidence based medicine. Additional recommendations included regular review of the categories as well as the need for future research.

If the experience of the last twenty years is an indication of future trends we can perhaps predict that indications for the use of IVIg will continue to expand and diversify. Thus there will be ongoing pressures on regulators and producers to meet the demands of the users.

Triggers and Targets

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Blood component therapy can save lives and improve quality of life however therapy is often given without reference to scientific evidence and ignoring potential risks. The NHMRC and ASBT are currently drafting guidelines on the indications for FFP. FFP in Australia is prepared usually from whole blood donations and currently is not virally inactivated. There are several international guidelines on indications for FFP although minimal scientific studies are available.

The main indications for use are:

- replacement of single factor deficiencies where a specific or combined factor is not available
- for immediate reversal of warfarin in the presence of potentially life threatening bleeding
- for treatment of coagulation deficiencies associated with DIC
- for treatment of TTP
- for treatment of bleeding with abnormal coags associated with bleeding
- for treatment of inherited deficiencies of coagulation inhibitors where a specific factor is not available

FFP is often used inappropriately both in terms of indications or dosage or correct group. Usage of FFP can be reduced by auditing records and having appropriate guidelines implemented.

New developments in unravelling neutrophil antigens

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Abstract not available at time of printing

Granulocyte Transfusions Renew Your Interest

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Renewed interest is proclaimed by most publications concerning therapeutic granulocyte transfusions and yet the practice is by no means universally accepted. The haematology community remains loosely divided into those who believe and those who do not. Granulocyte transfusions have enjoyed periods of popularity but the complexity of the many variables affecting efficacy and the faith of some proponents are likely equally to blame for the perceived lack of definitive evidence that the practice is truly beneficial. The importance of achieving adequate dosage of granulocytes is well appreciated and the optimal regimen of granulocyte colony-stimulating factor (G-CSF) and dexamethasone for donor stimulation has been well established. G-CSF increases component cell dose by a factor of 2- to 5-fold over corticosteroids alone in addition to inhibiting apoptosis of collected neutrophils and prolonging intravascular survival. The function of the collected neutrophils, including tissue migration appears well preserved and is not significantly diminished following storage for 24 hours. Donor availability remains an issue, however it is clearly feasible to recruit community donors rather than relying only on the traditional approach of using motivated family. Donors appear to tolerate the process without ill-effects, although there remain some safety concerns such as the relevance of splenic enlargement during G-CSF stimulation. G-CSF mobilised granulocytes are clearly then a feasible and practical therapeutic option that should be considered for all neutropenic patients. Definitive proof of clinical efficacy has, however, been difficult to come by. There are many Phase II clinical trials, usually with small patient numbers, multiple pretreatment variables and various clinical outcomes which attest to the efficacy of therapeutic granulocyte transfusion. In the setting of refractory neutropenic fever with or without proven fungal infection, most of us would contemplate using granulocyte transfusions, but at which time-point these should be commenced, with what sort of donor-compatibility testing, at what therapeutic dose and at what long-term risk to the donor remains unclear. Perhaps because of this uncertainty, the issue of prophylactic granulocyte transfusions is even less clear, however one remains tantalised by suggestions of improved survival in allogeneic stem cell transplant patients receiving HLA-matched granulocytes. It is quite possible that with the increasing sophistication of anti-cancer therapeutics, including molecularly targeted compounds and reduced intensity transplant conditioning, that granulocyte transfusions are destined to become an historical curiosity. Until that time, the current status of the practice and the evidence for and against clinical benefit needs continued discussion.

TRALI finding then firming a diagnosis

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Abstract not available at time of printing

Machine Collection

Neil Boyce
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Abstract not available at time of printing

Managing a cow of an issue a national approach to maintaining blood supplies

Carol O shea
ARCBS National

Abstract not available at time of printing

Vein to Vein: a virtual connection

Philipa Hetzel

Abstract not available at time of printing

Significant drivers of the plasma industry in Europe

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Following the development of processes to separate, purify and formulate individual proteins derived from human plasma, a number of medicinal products have and still are produced from this source material. For many years albumin was the main product of interest, followed by polyvalent and later specific immunoglobulins. After cryoprecipitate was discovered to be a rich source of clotting factor VIII, several clotting factor concentrates such as factor VIII, factor IX, prothrombin complex and subsequently protease inhibitors like C1 esterase inhibitor, antithrombin, and alpha-1-antitrypsin have been developed. Notably factor VIII, because of its low yield and instability combined with the increasing need for the optimal treatment of patients with hemophilia A, has been the driving force in many countries until recombinant factor VIII became available. The purification of immunoglobulin preparations led to the preparation of products for intravenous use (IVIG) which have shown to be safe and effective in the treatment of primary and secondary immunodeficiencies as well as various autoimmune diseases such as Kawasaki disease, idiopathic thrombocypenia, Guillain Barre disease and dermatomyositis. With the diminished the use of albumin and plasma derived factor VIII concentrates in some countries, IVIG has become (or may soon be) the new driving force.

The AIDS tragedy, notably for patients with hemophilia, had stimulated manufacturers to develop (and validate) effective and safe methods for virus inactivation during the fractionation process. Although several of these have been demonstrated to inactivate significant loads of enveloped viruses, there is room for improvement of the inactivation of non-enveloped viruses such as Parvovirus B 19. More recently, the potential threat of the transmission of prions by blood and plasma products has led to study the removal of these agents by nanofiltration.

Thus, depending on which aspect is discussed, a variety of drivers need to be considered: the logistics of the plasma supply, product safety, the fractionation technology, patient demands and product costs.

Molecular basis of blood group antigens and clinical applications

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We have come a long way since the discovery of ABO blood groups in 1900 when Landsteiner showed that the red cells of some of his colleagues were agglutinated by the plasma of others. Advances in the understanding of the nature of blood group antigens and the molecules on which the antigens are expressed have been facilitated by technical improvements in the methods for protein sequencing and for analysis of DNA and RNA. A molecular approach to the nature of blood group antigens provides information about antigen structure and the genetic mechanisms giving rise to the structural diversity at the cell surface.

Blood group antigens are inherited amino acid sequences or carbohydrate motifs that give rise to polymorphic characteristics on the red cell surface. The majority of antigens are located on proteins which pass through the red cell membrane once (eg Kell, GPA), or multiple times (eg Rh, band 3), or are attached by a glycosylphosphatidylinositol anchor (eg Cromer, Yt). Others are carbohydrate residues attached to proteins or lipids as defined by specific glycosyltransferases(eg ABO). More that 250 antigens have been defined and placed into 23 blood group systems.

The cloning of blood group genes has led to the determination of the molecular basis of many blood group antigens. Clinical applications include the prenatal determination of a fetus at risk for HDN, the genotyping of recently transfused patients, the genotyping of patients with a positive direct antiglobulin test and the determination of the origin of lymphocytes in transplant recipients.

Scientific secrets of dendritic cells

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Abstract not available at time of printing

RhD antigen expression: what can affect it?

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The RhD antigen is one of the most immunogenic of all human blood group antigens and is of major clinical significance because of its association with haemolytic disease of the newborn and with delayed transfusion reactions. Red cells are typically typed as positive or negative depending on whether the RhD antigen is present or absent. The RhD negative trait arises in about 16% of white Caucasians because the RhD gene was completely deleted at some time point in human history. Variations in the expression and or presence of the RhD antigen may also arise from a variety of genetic alterations as described here.

Qualitative changes in the RhD antigen typically may arise from exon rearrangement between the RHD and the closely linked and homologous RHCE gene. The resultant range of hybrid Rh D-CE-D proteins contains some but not all of the RhD epitopes producing the range of RhD variants. These may be immunogenic and of clinical significance.

Very rare mutations in another structural gene, the RH50, can prevent the insertion of normal Rh D and CE proteins into the red cell membrane, producing the Rh null trait . This is associated with abnormal cell shape and mild haemolytic anaemia. We have described a unique heterozygote case with unusual mutations in both exons and intron regions of the RH50 gene. These studies show that the RH50 gene, encoding the Rh50 glycoprotein, is essential for the correct assembly of Rh D and CE proteins into the red cell membrane.

However variations in RhD antigen expression can be quantitative and associated with reduced level of expression phenotypically referred to as weak D . The weak D antigen does not lack any of the epitopes comprising the RhD antigen mosaic. It is now known that weak D types are associated with mutations in the RHD gene. In a study of weak D samples found during normal serological testing we found that 96% of weak D samples carried a mutation in the RHD gene sequence. In general the mutations reside within the hydrophobic region of the D protein and not within the extracellular domains. These mutations may be responsible for reducing the level of efficiency of insertion of the RhD protein into the red cell membrane.

Alterations in the highly conserved genetic sequence of the Rh blood group system often result in reduced antigen expression. The increasing number of new mutations discovered leading to a reduction in antigen expression highlight the importance that small changes in RH gene sequences play in the assembly of the Rh protein into the red cell membrane.