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New trends in blood transfusion

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INTRODUCTION

I sometimes wish I had a crystal ball to look into the future and predict what the trends in blood transfusion will be over the next 10-20 years. If I had been writing this article 20 years ago, I am sure that I would have never imagined some of the developments that have taken place in blood transfusion over recent years. For example, who would have anticipated that AIDS would make its debut with its significant effects on donor recruitment and blood usage?

Blood transfusion in clinical medicine has experienced significant changes in recent years and rapid developments continue to take place on many fronts. Numerous factors will determine the future use of blood and blood derivatives such as:

1) The tendency towards a more rational use of blood and blood components for those patients who really need them. Education of clinicians on the proper use of blood is now becoming an accepted aspect of medical training. Responsible clinicians are re-examining the benefit-to-risk relationship of blood transfusion. However, there is a great deal of ground to be covered since many clinicians consider blood and blood components on the same level as any drug that they prescribe. In some countries, the establishment of Hospital Transfusion Committees has helped a great deal towards a more rational use of blood and it is expected that such committees will be established in more and more hospitals worldwide.

2) Transfusion-transmitted infections and their prevention. The knowledge that HIV infection can be transmitted by blood transfusion has made clinicians and the general public realise that blood transfusion can be dangerous. Some advocate the implementation of a «no risk» policy and would encourage testing for agents that can be only remotely or theoretically transmitted by blood transmission in some countries. For example, at present it would be inadvisable and uneconomical to screen blood donations for anti-HTLV I and for anti-HIV-2 in the UK; however, some of our colleagues would be happy to introduce such screening.

Large-scale techniques to screen for viral contamination in donated blood and plasma are improving in specificity and sensitivity. For blood donors, with a low prevalence of most infectious agents transmissible by blood transfusion, high specificity is of paramount importance; false positive results with the subsequent information to a negative donor that he/she is seropositive for HIV would have devastating consequences. Not only are the specificity and sensitivity of screening tests improving, but so are the confirmatory tests with the introduction of new technologies such as polymerase-chain reaction. In addition, a spin-off of the threat of transfusion-transmitted infections has been the progress of methods for the inactivation of viruses in fractionated blood products.

3) Cost. In these times of financial constraints, where clinicians are asked to question their practices and become «cost-effective», those who prescribe blood are starting to look into cheaper alternatives to blood and into the appropriate use of component therapy.

4) New technologies for storage systems and preservation have allowed prolonged storage time and improved the function and survival of stored red cells and platelets.

5) Advances in surgery and anaesthesia have decreased the use of blood and components for numerous procedures. For example, the volumes of blood used at present for coronary artery bypass surgery bear no resemblance to the volumes used 10 years ago for the same procedures.

6) Molecular genetics with the advent of genetically engineered Factor VIII and monoclonal antibodies for use in the immunohaematology and microbiology laboratories and for potential clinical use (eg anti-HBs, anti-Rh).

7) Scientific developments have opened the possibility of producing cellular elements *in vitro* some time in the future.

8) Developments in the physicochemical nature of filtration membranes are likely to enable the removal of specific proteins or toxins from plasma without the need for plasma exchange. Methods of modifying artificial membranes with attachment of ligands for specific depletion of a particular substance are being actively developed. For example, Prosorba, a small disposable filter containing a molecule that binds to immune-blocking

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factors, is being tried on patients with AIDS and with cancer.

9) The expansion of the practice of autologous transfusion in its various forms of pre-deposit transfusion, pre-operative haemodilution and intraoperative salvage, is a reality for elective surgery in those developed countries who can afford such practices and especially in those countries where the dangers of transfusion-transmitted infection are significant.

10) Progress in automation and computerization is enabling positive sample identification in addition to record-keeping and stock control. Improved computer technology will allow the linking of crossmatched blood components to patients.

11) Laboratory testing capability has been enhanced and improved due to automation and improved techniques such as solid phase serology and flow-cytometry.

12) Investigation of the causes of transfusion-induced immunosuppression and its clinical consequences, if any. Whether such immunosuppression influences the risk of cancer recurrence and possibly also of infection is a matter that requires further investigation. Meanwhile, approaches to reduce the immunosuppressive effects of blood transfusion are being developed.

It is unfortunate that politics and the lay public are interfering with the decisions of transfusion services and in particular in those related to screening tests for infectious agents. We should remember that the decision to implement testing of blood donations for anti-HIV in the USA and some other countries as soon as mass screening tests were approved by the FDA, was not a clinical one but a decision forced by panic exploited by the media. The problems encountered in the first year by the precipitous introduction of anti-HIV testing in the USA were mainly due to the lack of time to implement rational and properly planned testing (no Reference Laboratories, no provision for counselling, no alternative testing sites, no freely available clinics for sexually transmitted diseases). A trend has been started and it will be difficult now to revert to the times when the implementation of laboratory test on blood donations was based on strong scientific evidence. For example, at present in the USA, blood donations are tested for ALT and anti-HBc as «surrogate» markers for non-A, non-B hepatitis (NANBH). Now that a test system has been devised for HCV, supposedly the main agent for post-transfusion NANBH, it is highly likely that transfusion services in the USA will test for anti-HCV *in addition* to surrogate screening because the public would not allow controlled clinical trials to see what the level of post-transfusion NANBH is at present in the USA and whether any or all of these tests will decrease its current incidence.

Consumer demands are governing many aspects of transfusion medicine. It is the consumer who is often pressing for autologous and «directed» donations as well as for non-specific screening tests, for the production of Factor VIII by recombinant DNA, etc. due mainly to the irrational fear of AIDS.

With the advent of the Consumer Protection Act, 1987 in the UK and the European Community Product Liability Directive, at the beginning of 1988, blood and blood derivatives will no longer be considered a service but products. These regulations create a regime of strict civil liability for damage caused by defective products in all member countries of the European Community. Hence, recipients of blood components or blood products will be able to sue manufacturers (ie Transfusion Centres or fractionators) without the need to prove fault. This means that European countries will now be in a worse position than most states in the USA where litigation has been passed to consider blood a service and not a product. Transfusion Services will have to spend more energy and resources to comply with regulations of good Manufacturing Practice in order to avoid producing defective products. Such measures will not provide a defence under the new regulations but will probably reduce the likelihood of claims and will increase the confidence level of in-house manufacturing activities.

Quality Monitoring and Quality Assurance Departments with Managers independent of Production and in charge of the ultimate release of blood components have now become essential. Not only will we need to perform adequately but we will need «to show that we are performing» according to new regulations. Every aspect of the work in a Transfusion Centre and in a hospital blood bank will require a serious reappraisal and will need to be documented according to up-to-date written Standard Operating Procedures. Both internal (including «in process») quality control and external quality assessment will need to be formally organised under a designated and independent Q.A. officer with supporting staff who will be respected by colleagues throughout the Transfusion Service. It is possible that the laws on Product Liability will stimulate the filing of law suits for defects in the product. Hence, European countries will need to have nationally agreed guidelines or requirements on specifications of blood components and blood products and on all the procedures performed by Blood Transfusion Services according to realistic criteria. Such guidelines will enable the defence to establish that all procedures have been performed according to nationally approved recommendations, and that the state of scientific and technical knowledge at the time of supply of the product was not such as to enable the existence of the defect to be discovered. The capacity to avoid liability or defend an action will depend to a great extent on the maintenance of clear, accurate and comprehensive records relating to the procurement, use, modification, processing and supply of products. Transfusion Centres will need to record clearly from whom and when equipment used in production, such as plastic packs, was obtained. Details such as the supplier, serial and batch number, delivery date, date of usage, etc, will need to be recorded. Since an obligation arising from liability is extinguished only after a period of ten years and up to one year is allowed for the serving of a writ, such records should be kept for a period of eleven years. In this aspect, computerization and data processing will play a major role. The same regulations on record keeping apply to the administration of blood derivatives; the ultimate fate of each unit of blood, blood component or blood product must be known and records should be kept for 11 years.

What are the tendencies for the future use of blood derivates? It is likely that the use of whole blood and red

cell components will remain the same, that the use of platelets will continue to increase, that the use of albumin and fresh frozen plasma will decrease, that the use of Factor VIII and Factor IX will remain the same or increase slightly, that intravenous immunoglobulins will be used more and for more procedures. In addition, we are already seeing and will see more auditing of the fate of blood and blood components with classification of recipients of blood by disease. Doctors and patients are questioning the need and safety of blood transfusions.

RED CELLS

It has been estimated that the requirements for red cells of a country can be met if the rate of blood collection is 50 per thousand of the population. In England and Wales, the average rate is approximately 40 per thousand and this is barely sufficient. But demands within one country vary depending on the number of specialised and teaching hospitals. For example, despite the high rate of blood collection of 55 per thousand at our Centre, there are times of the year when we have to ask for help with red cell components from other Regional Transfusion Centres due to the high blood usage of London hospitals.

Several strategies are being devised to decrease red cell transfusions and to diminish the risk of transfusion-transmitted infection such as acceptance of lower levels of haemoglobin (Hb) by surgeons, obstetricians and anaesthetists, autologous transfusion, use of erythropoietin, DDAVP and aprotinin, development of red cell substitutes and of methods of sterilization of blood. Despite the progress in alternatives to red cell transfusions that might reduce the need for homologous blood, blood collected from carefully selected voluntary donors will continue to be the therapeutic mainstay for the foreseeable future. Unfortunately, due to the unpredictability of demands and often of donor attendance, and due to the need to keep an emergency reserve, there will always be a proportion of donated red cells that will need to be discarded.

1) The requirement of a predetermined Hb level of 10-11 g/dl or a haematocrit of 30 before surgery has no scientific data to support it. Recent data have shown that patients can safely undergo general anaesthesia and surgery with Hb levels of 7 g/dl or less provided cardiac output, normovolaemia, an appropriate inspired O_2 concentration and normal tissue perfusion are maintained. More and more surgeons are abandoning the practice of transfusing red cells to treat hypovolaemia or to promote wound healing. Progress in anaesthetics and surgical techniques is allowing a better control of surgical bleeding.

2) Pre-deposit autologous transfusion is recommended for patients undergoing elective surgery in whom the need for blood transfusion is anticipated according to the transfusion policy schemes of the surgical team concerned. In certain countries and in some states of the USA, pre-donation schemes are rapidly expanding; however, current experience with autologous transfusion is limited to around 2 % of transfusions in the USA. On the other hand, it is unlikely that schemes for the long term storage of autologous blood in the frozen state will flourish; such schemes will only benefit the very rich who will only donate blood for themselves.

3) Acute normovolaemic haemodilution has become popular with some surgeons who claim that it decreases homologous blood requirements, provides a source of fresh whole blood with intact platelets for transfusion at the end of the operation and improves tissue perfusion through beneficial rheological alterations associated with haemodilution.

4) With modern cell savers, blood can be salvaged from the operative site or chest tubes and reinfused to the patient after prompt and proper processing. Blood salvage is being used increasingly in those operations when it is anticipated that at least 1.5-2 litres of blood will be salvaged (eg liver transplantation, ruptured aortic aneurysms). It is possible that the cost of equipment and disposables will decrease in the future, thus making intraoperative salvage a more realistic proposition for massive transfusion.

5) Deliberately induced hypotension intraoperatively is another approach which is being developed to reduce blood loss during some surgical procedures.

6) Human recombinant erythropoietin reduces blood transfusion needs by not only inducing a progressive rise in haematocrit but also a pronounced shortening of bleeding time in patients with chronic uraemia. Several groups are already treating patients with end-stage chronic renal failure with erythropoietin and are compromising at lower haematocrits in order to avoid thrombotic complications and hypertension while still improving haemostasis and abolishing symptoms of anaemia. Trials have already started on the use of erythropoietin for other forms of chronic anaemia. In addition, it has been suggested that administration of recombinant erythropoietin prior to autologous blood donation will increase the amount of autologous blood that can be collected in a short period of time. Others are proposing the use of erythropoietin post-operatively.

7) Aprotinin (Trasilol), a serine proteinase inhibitor, in high doses has been shown to reduce blood loss considerably in patients having repeated open-heart surgery through a previous sternotomy wound and in patients with septic endocarditis. Although the exact mechanism of action of aprotinin is unknown, it seems to protect platelets from pathological activation in the extracorporeal system while maintaining their responsiveness to physiological challenge, Clinical trials are under way on the use of aprotinin on patients undergoing repeated cardiac surgery; preliminary information suggests that this drug can abolish the need for homologous blood transfusion in more than 50 % of such patients.

8) DDAVP or desmopressin acetate, when given to patients undergoing cardiac surgery, has been shown to reduce bleeding. Although it is known that this drug increases the plasma concentration of Factor VIIIC and von Willebrand factor, the exact mechanism of action in cardiac suegery patients is unknown. Some cardiothoracic surgeons have incorporated DDAVP to their routine procedures and it is likely that others will follow.

9) Although considerable progress has been achieved in recent years, the development of clinically useful red cell substitutes capable of transporting oxygen has not met with the success expected a few years ago and no product is available for general use. The ideal red cell substitute should have the following properties: O_2 dissociation curve the same as that of normal red cells; no toxicity or immunogenicity; normal flow properties; adequate persistence in the circulation; devoid of typing and crossmatching requirements; long storage time or shelf-life; stability at ambient temperature and low cost. Three types of red cell substitutes are being developed: perfluorcarbon emulsions, haemoglobin solutions (tetramers, polymers, in haemosomes) and synthetic chelates.

(i) The perfluorocarbon (PFC) emulsion Fluosol-DA is of limited use due to its short lifespan in the circulation and the need for concurrent high inspired oxygen- concentration. It apperars to be ineffective in severe acute anaemia. PFCs are insoluble, very unstable and require oncotic agents such as hydroxyethyl starch (HES) for their administration. Other unfavourable effects include the inhibition of phaghocytosis; inhibition of chemotaxis, adhesion and migration of neutrophils; alteration of the coagulation mechanism and activation of complement. At present, and in the near future, the use of PFCs is restricted to CO intoxication, organ perfusión, some cases of extracorporeal circulation, ischaemia and oxygen delivery to tumours.

(ii) The stroma and lipid-free Hb solutions, when at the same oncotic pressure as plasma, have lower capacity of carrying O_2 than red cells and have a considerably shorter half-life in the circulation. HB tetramers in solution quickly dissociate into dimers with loss of 2,3-DPG and high O_2 affinity. In experimental animals they have vasoconstrictive activity with temporary alteration of renal function. Nephrotoxicity seems to be the main obstacle to future clinical trials of HB solutions. Unfortunately, studies in experimental animals, except baboons, cannot predict renal dysfunction in humans. Studies on the effect of HB solutions on the immune system with special reference to immunosuppression will also need to be undertaken before doing such clinical trials in humans.

Polymerized Hb linked to piridoxal phosphate plus a carrier (Poly SFH-P) is another compound which is being developed experimentally. However, the coupling is not homogeneous, the compound has a normal Hb concentration of 15 g/dl but slightly less affinity for O_2 with a P_{50} of 20-22 torr (normal = 26 torr) and the half-life in the circulation is approximately 24-48 h.

Work in Amsterdam with NFLP (2-nor 2 formil piridoxal 5'phosphate) which interlinks with β chains of Hb has shown that it decreases the Hb affinity for O_2 and increases the retention time in the circulation. NFLP gives the molecule greater stability without dissociation into dimers and this type of tetramer does not seem to have greater viscosity or nephrotoxicity as other forms of Hb. The lifespan in the circulation has been shown to be 8-10 days. HbNFPLP can be safely stored at 4 °C for at least six months.

A novel approach which deserves further investigation is the development of haemosomes or stroma-free Hb encapsulated in phospholipid bilayers (liposomes) containing cholesterol, dicetylphosphate and probably vitamin E as a stabilizer. The advantages of haemosomes are their biodegradable membrane, acceptable concentration of HB, acceptable O_2 affinity, free passage through capillaries and absence of complications such as D.I.C. or immediate nephrotoxicity. The disadvantages of haemosomes are their short half-life which varies from 5.8-15 h depending on the method of preparation, their irreversible binding to tissues with its unknown consequences and the inability to prepare large volumes of suspensions with reproducible, uniform particle size distribution which will alow sterilization by filtration. In future, the long term effects of haemosome infusions will need to be investigated and their half-life will need to be improved, perhaps by reducing and standardising the particle size distribution.

10) Therapeutic advances are likely to reduce the number of transfusion-dependent patients in future, thus reducing problems of alloimmunization. In Europe and in Seattle, patients with aplastic anaemia are being transplanted before they become sensitized by transfusion. In Italy, young thalassaemic patients are being transplanted with HLA-identical sibling grafts with disease-free survival of 76 % at 5-57 months.

11) Preservatives and additive solutions: It is anticipated that those countries not using large volumes of red cells in additive solutions (SAG-M, ADSOL etc.) will increase their usage to at least 60 % of the blood collected. It is my personal opinion that there is still a place, albeit small, for whole blood for the massively transfused patient who does not require unnecessary exposure to red cells in additive solutions plus fresh plasma and/or 5 % albumin.

With reference to the shelf-life of red cells, the use of ADSOL has already increased it to 49 days and possibly 56 days. It is not envisaged that a longer storage time will be required.

12) There is a great stimulus for the development of cellular substitutes of blood due to their high demand, specially non-immunogenic, non-virally-infected red cells and platelets. Advances in biotechnology might make it possible in the future to grow pluripotent stem cells and stimulate them to divide and differentiate into mature red cells, platelets and granulocytes. Cultures of human bone marrow cells have only met with partial success; whilst some differentiation has occurred, not all cell lines have behaved in a similar manner. Numerous factors are required to act together in the culture of pluripotent stem cells such as feeder cells, semi-solid matrix, growth factors and culture medium with aminoacids, vitamins, serum, electrolytes, glucose, hormones, etc.

13) One of the major hazards of blood transfusion is still the possibility of microbial infection. Research is being undertaken in the USA adding Psoralin in vitro to the units of blood. So far, the results look promising with inactivation of all types of viruses and bacteria. It remains to be seen whether it will be safe to administer

Psoralin to patients in every unit of blood or blood component they receive.

GRANULOCYTES

The trend shows that their use will continue to decrease; the indications for their use are very limited in this era of modern antibiotic therapy. Since long-standing severe neutropenia is a major cause of often severe and even of fatal infection in patients with cancer, a shortening of any neutropenic period is to be desired. Urinary and recombinant human granulocyte- or granulocyte/macrophage-stimulating factors (G-CSF-HU and rHuGM-CSF) have been used successfully in bone marrow transplant (BMT) recipients causing an accelerated myeloid recovery and significantly reducing the duration and severity of granulocytopenia after transplantation. In addition, the function of mature granulocytes is stimulated by these factors. It is likely that in the future, these haematopoietic growth factors will be developed further and become devoid of their toxic side effects such as myalgia and fluid retention. It is hoped that a combination of growth factors will contribute synergistically to accelerated BM recovery.

Methods to prolong, to 24h, the storage time of granulocytes concentrates collected by apheresis are being developed.

In the 1970's, there was a place for the mass collection of buffy coats from blood donations for the extraction of interferon alpha. But this product was only 1 % pure and contaminated with lymphokines. Now, the 3 types of interferon: alpha, beta and gamma, can be produced with high purity by recombinant DNA technology and there is no need for buffy coats. Interferon alpha, the more widely used, can be produced by lymphoblastoid cells cultured in vitro or by recombinant DNA technology in *Escherichia coli.* The main indications for alpha interferon are very few: hairy cell leukaemia, chronic granulocytic leukaemia, Kaposi's sarcoma and condylomata acuminata. Some success has been obtained with interferon therapy for hepatitis B and hepatitis non-A, non-B. The future of interferons seems to be mainly as an adjunctive therapy to other anti-tumour drugs and invitro studies of tumour growth and function of macrophages, T lymphocytes and Killer cells.

PLATELETS

Platelet transfusions can only continue to increase due to numerous factors such as a) increase in the number of bone marrow transplants (BMTs) to treat conventional conditions such as leukaemia and aplastic anaemia as well as other conditions which were previously treated with more conservative therapies (eg. thalassaemia, certain congenital metabolic deficiencies, etc). b) New technologies in BMT such as T-cell depletion that prolong the period of thrombocytopaenia. c) More agressive chemotherapy in the treatment of malignancy. d) Realisation that non-surgical bleeding after massive transfusion or extracorporeal circulation is more often due to thrombocytopaenia than lack of coagulation factors, etc.

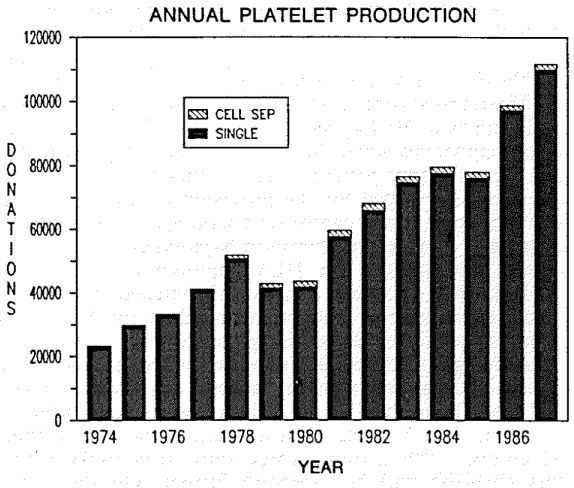

Fig. 1. Annual production of platelet concentrates by the North London Blood Transfusion Centre. Single = platelet concentrates prepared from routine blood donations within 6 hours of collection. Cell sep = platelet concentrates collected by apheresis (most Fenwal CS-3000 and from Haemonetics-50 surge) mainly for the treatment of patients who have become refractory to the transfusion of random platelets.

Unfortunately megakaryocyte-colony stimulating factors (CSF) have not met with the success obtained with granulocyte-CSF. It is hoped that the time will come when the period of thrombocytopaenia post-BMT will be shortened.

At our centre, the number of platelet concentrates processed has increased from approximately 22,000 in 1974 to more than 112,000 in 1987 and in 1988 the figures nearing 120,000 (see Table 1). The major users are 4 active bone marrow transplant centres and a large oncology unit. Incidentally the number of platelet concentrates collected by cytapheresis has not increased because we limit their use to patients who have become refractory to the transfusion of random platelets.

Steps to minimise the problem of refractoriness, mainly due to alloimmunisation of recipients to HLA antigens are being adopted and developed by many transfusion centres. The steps that can be adopted are as follows:

- reduce the white cell contamination of random donor platelet concentrates to a minimun by careful processing and, if necessary, an additional centrifugation step.
- filter the platelet concentrates through specific white-cell depletion filters (e.g. Pall, Asahi).
- use single-donor platelet concentrates prophylactically. This step has not been shown to definitively reduce the incidence of HLA-alloimmunisation. Platelets collected by apheresis are expensive but, in general, are less contaminated with white cells.

Experimental approaches to reduce HLA alloimmunisation consist of:

- Use of UV light irradiation to inactivate HLA class II antigens. The major obstacle to this approach in humans is the development of a plastic that will allow the passage of UV light.

– use of systemic cyclosporine in conjunction with the above or of cyclosporine-loaded platelets.

– induction of tolerance by the injection of soluble HLA class I antigens.

Most transfusion centres have seen the need to recruit panels of HLA-typed donors who will volunteer for thrombapheresis when the need for HLA-matched platelets for refractory patients arises. Numerous approaches are being developed for the provision of such platelets. For example, at our centre we encourage the enrolment of platelet donors with common haplotypes as well as of donors homozygous for HLA haplotypes (e.g. A1-B8, A2-B7). We also use donors mismatched for cross-reactive antigens or donors mismatched for HLA-B44 which is poorly expressed on platelets.

Although some of the new permeable plastics will maintain the platelets functional for 7 days, most centres will limit their shelf-life to 5 days due to the danger of infection with bacteria such as salmonella and shigella which grow well at room temperature. It is unlikely that this shelf-life will be extended further. On the other hand, due to the poor recovery, the use of frozen platelets will be limited to the provision of HLA-matched platelets when fresh platelets are unavailable or to the provision of platelets of rare types such as P1 (A1 negative) for neonatal transfusion.

Prostacyclin is being used experimentally to reduce the activation of platelets in vitro.

Artificial platelet-suspending media are being developed. If this approach becomes a reality for routine transfusion therapy, the volume of plasma saved for fractionation will be considerable. For example, at our centre, if we collect annually 120,000 platelet concentrates from routine donations, and since each platelet concentrate contains 50 ml of plasma, the saving of fresh plasma for fractionation will be of the order of 6,000 kg.

Some workers have tried to produce artificial platelets but, so far, they have met with limited success. Platelet substitutes prepared from soybean phospholipid have failed to control bleeding and so have freeze-dried and fragmented platelets. The major platelet glycoproteins have been encapsulated in liposomes and although some aggregation has been obtained, there is still the difficulty of identifying all the proteins required for platelet function and the mechanism for the correct orientation of these proteins in the outer layer of the liposome. It looks as if we are still far from the ideal platelet substitute and it is impossible to forecast, at this time, whether the liposome approach will meet with success.

PLASMA AND PLASMA PRODUCTS

The World Health Organisation, the Council of Europe and the International Society of Blood Transfusion have recommended that all countries should aim towards self-sufficiency in blood and blood products. Although most countries are able to supply their own needs for blood and blood components, the majority are unable to meet their demands for fractionated plasma products, and specially of Factor VIII. The minority of countries in the world have plans and realistic programmes to achieve national self-sufficiency in plasma. It is expected that more countries will have such plans in the future.

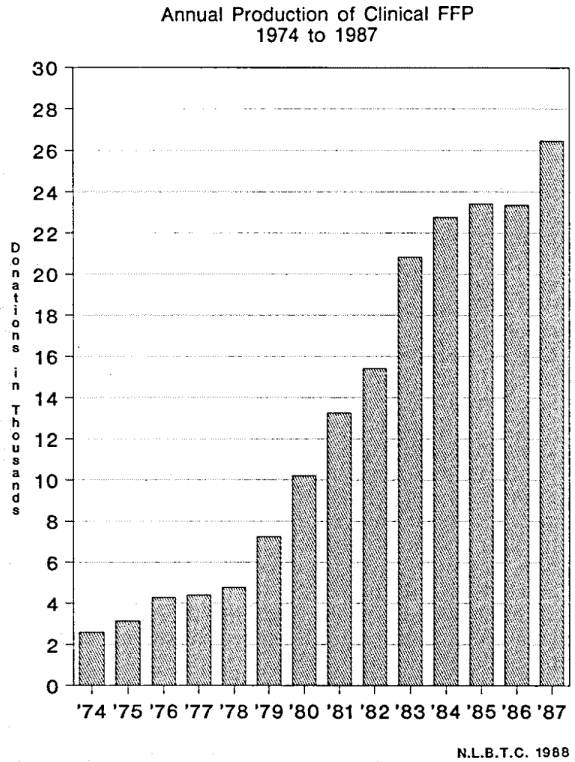

Fig. 2. Increasing annual production of fresh frozen plasma for clinical use, separated from routine blood donations from 1974 to 1987.

It has been estimated that 10,000-12,000 kg (or litres) of plasma per million of the population are required to achieve self-sufficiency in fractionated plasma products. It is possible that these figures are higher now with the decreased yields of Factor VIII from fresh plasma due to chemical and prolonged heat-inactivation of viruses in blood products. If we accept that 50-55 blood donations/year/million of the population is the optimum, then 5,000-6,000 kg plasma/year/million of the population can be achieved with a 50 % separation rate into SAG-M or ADSOL; 7,000-8,400 kg of plasma can be collected when SAG-M or ADSOL; 7,000-8,400 kg of plasma can be collected when SAG-M is used in order to remove plasma from 70 % of donations. The shortfall in plasma to achieve self-sufficiency will be therefore of the order of 3,000-7,000 kg depending on the rate of separation of whole blood and on the targets set for each country. There are two options to cover this shortfall: (i) increase blood collection to obtain a sufficient volume of plasma. This option, although cheap, leads to an unnecessary waste of red cells which is unethical and unacceptable. (ii) collect plasma by apheresis, a more expensive option in view of the high cost of disposables. Some countries have already introduced the wide use of machine plasmapheresis on a paid or voluntary basis. In the UK we have shown that voluntary plasmapheresis is a real

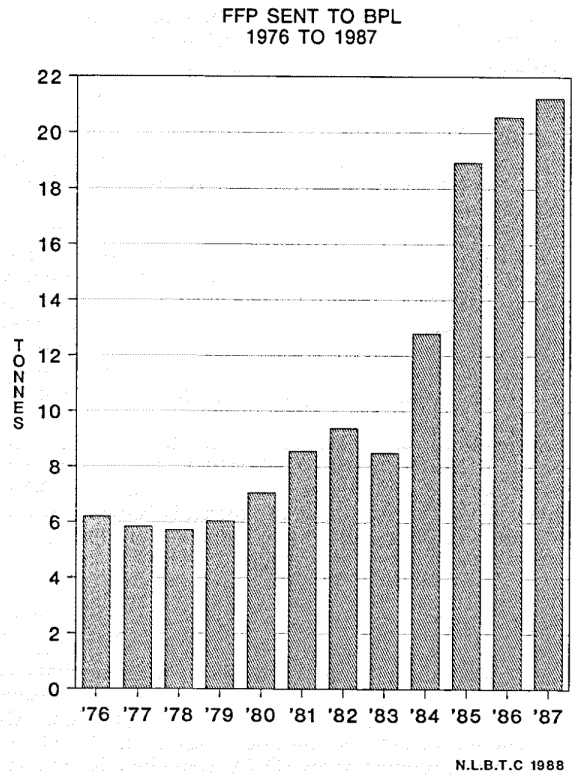

Fig. 3. Annual production by the North London Blood Transfusion Centre of fresh frozen plasma (FFP), in tonnes, for fractionation by the Blood Products Laboratory. Most of this plasma up to now is recovered from routine donations and the rest is collected by automated plasmapheresis.

possibility. Research work is currently under way to show whether plasma collected by filtration apheresis (eg Baxter Autopheresis machine) is as satisfactory for fractionation as plasma collected by centrifugation apheresis (eg Haemonetics PCS machine).

There is a conflict of interests between plasma required for fractionation and the clinical requeriments for fresh frozen plasma (FFP) and platelet concentrates (50 ml plasma/concentrate; see above). Despite efforts from specialists in blood transfusion to induce clinicians to use less FFP, it is stille being abused. For example, demands for FFP at our Centre are increasing annually despite continuous education (see Table 2). Because of this high demand and the increasing demands for platelets, we are limited in the amount of plasma that we can recover for fractionation (Table 3). The problem of abuse of FFP has been addressed by the NIH in a Consensus Conference.

In England and Wales, the plans are to achieve self-sufficiency in plasma products by 1990/91. For this purpose, the Blood Products Laboratory (BPL), a new fractionation plant, has recently been built at a cost of £ 62,000,000. We are already self-sufficient in immunoglobulins for i.m. use including anti-Rh and in Factor IX but we are still importing large amounts of Factor VIII and albumin. We expect to achieve self-sufficiency with a national plasma collection rate of at least 550 tones/year or 11 tonnes/million population. The national collection rate of 2.1 million blood donations/year will yield approximately 350 tonnes of fresh frozen plasma if 70 % of donations are processed and if plasma for clinical use is substracted. The gap of 220 tonnes of FFP will need to be collected by apheresis.

The national strategic plan is that each Regional Transufsion Centre will need to collect plasma for fractionation according to its catchment population. For our Centre, with a population of 3.4 million, the annual target for 1988/89 is 31 tonnes of fresch plasma and for 1989/90 the final target is 38 tonnes. Our plans are to collect about 60-70 % of our blood intake into SAG-M and to increase plasma collection by machine apheresis. For this purpose, we have acquired 3 machine plasmapheresis clinics with a total of 33 machines (plus spares). The problems with the collection of plasma by apheresis from voluntary donors are its high cost and, specially, donor recruitment. Although the clinics are well attended at certain peak hours, it is difficult to encourage regular attendance during working hours. Publicity will need to be geared to the recruitment of those plasma donors who can attend during slack periods in order to achieve the ideal collection rate of 1 tonne of plasma per machine per year.

If the Blood Products Laboratory fractionates 550 tonnes of plasma/year, the notional transfer value of the issued products will be of the order of £35,000,000 or more. This means that the capital spent in the building of BPL will be recouped in just a few years.

Factor VIII

Despite the sad fact that a high proportion of severe haemophiliacs in the developed world have acquired HIV infection and AIDS, it is not envisaged that the use of FVIII will decrease. With requirements of FVIII of 30,000 to 250,000 units/patient/year and with an incidence of haemophilia A of approximately 1 in 10,000 of the population, the universal requirements of FVIII have been estimated at approximately 500-800 grammes of protein.

More money will be spent in the new, safer Factor VIII concentrates such as those purified with the aid of monoclonal antibody columns or those treated by prolonged heat and/or chemicals (e.g. solvent-detergent TNBP/Triton) to inactivate viruses. Research will continue to achieve the best methods of removal or inactivation of contaminating viruses with a minimun loss of FVIII. Currently there is a significant loss of FVIII activity during purification and viral inactivation; on average only 10-20 % of the starting activity in plasma is retained in the final concentrate. Although the main cause for concern is contamination with HIV-1, HBV and hepatitis non-A, non-B virus, there is growing concern about the possible presence of other viruses. Additional aspects that will continue to receive the attention of the manufactureres are: a) the development of concentrates that will not stimulate antibodies to FVIIIC, b) the production of concentrates devoid of degradation fragments responsible for immunosuppresive effects, c) the development of methods to increase the circulating levels of FVIII both in donors and patients. Although

DDAVP has proved successful in the treatment of mild and moderate haemophilia, the increase of circulating FVIII in blood donors does not seem to be recovered during fractionation, and d) the study of anticoagulants (e.g. heparin) that might improve the yield of FVIII in plasma.

One of the major problems in the production of FVIII by recombinant DNA technology is large-scale synthesis with purification methods that will not inactivate the protein and/or alter its function. Although limited amounts of recombinant FVIII produced in cultured mammalian cells are available at present, we anxiously await the results of the clinical trials which have already started. Preliminary data, derived from the administration of rDNA FVIII to severe haemophiliacs suggest that it is a safe and effective therapeutic product with a biological half-life of 15h and no side effects so far. Since the amount of FVIII required to treat the haemophilia patients of the world is relatively manageable (e.g. approximately 280-300 g of pure protein are required to supply the 500 million units needed to treat all the haemophiliacs in the USA), we should expect that within the next 10 years methods to produce large volumes of rDNA FVIII will be available. However, we do not know when rFVIII will be available or what its cost, relative to fractionated Factor VIII, will be.

Since the batch fermentation of cultured animal cells is an expensive and technically complicated procedure, the idea of producing such proteins in recombinant or «transgenic» animals is being investigated. Techniques for the introduction of foreign genes into the germline of animals have recently been developed. It has been possible to direct the synthesis of a particular protein (e.g. FVIII) to a specific tissue (e.g. mammary glands of goats) and this methodology may have the potential in future of being applied to the production of human plasma proteins from farm animals (e.g. FVIII-rich milk).

The ultimate word in FVIII has not been said; research is still ongoing in order to produce an improved product by, for example, deleting segments of the protein without biological function or producing molecules devoid of immunogenic properties.

Factor IX

Requeriments are much lower than for FVIII but there is still a need for plentiful supplies of concentrates devoid of thrombogenicity and of viral contamination. There is no need for increased world supplies of Factor IX since the capacity to produce more concentrates is available in the plasma used for the production of FVIII. The cDNA for Factor IX has been cloned but, so far, no FIX concentrates have been produced by recombinant DNA technology.

Albumin

Its global use seems to be decreasing recently mainly because large users such as Japan and Germany are being more selective in its clinical indications. Nevertheless, it is estimated that between 100 and 300 kg of albumin/million population/year will continue to be used in developed countries. Albumin is devoid of problems of transmission of viral agents due to the pasteurization process used in its manufacture.

Although the cDNA for albumin has been available for a long time and the expression of genetically engineered albumin has been accomplished in *E. coli,* for the time being, the production of rDNA-albumin does not seem to be a financially viable proposition. The volumes of protein required to treat a single patient are so large that at the present time it is considerably cheaper to obtain albumin by plasma fractionation. This high cost is due to the very large *E. coli* fermentation capability required to meet the worldwide demand; 193×10^6 litres of fermented culture would be needed. It has been estimated that 10 billion gallons of water would be needed to produce from *E. coli,* the albumin currently being fractionated from plasma using only 50 million gallons of water. From these estimations, it has been calculated that the cost of albumin produced by recombimant DNA techonology would be at least double the cost of albumin currently fractionated from plasma. Other systems, such as *B. subtilis,* are currently being investigated for a more cost-effective production of albumin.

Fibrinogen

At present, its only source in therapeutic amounts is from cryoprecipitate since no safe, virus-fre fibrinogen concentrate has been produced by fractionation. Although the cDNA for fibrinogen has been cloned, its commercial production by rDNA technology is not contemplated.

Other proteins

The cDNA for alpha-1-antitrypsin, antithrombin III, protein C and plasminogen activator have all been cloned. Due to the small clinical demands at present, commercial companies do not consider the production of these genetically engineered proteins a priority although clinical or pre-clinical trials of some of these recombinant products have already started.

Immunoglobulins (Ig)

The use of intravenous broad spectrum and specific immunoglobulins is rapidly increasing for the treatment of hypogammaglobulinaemia, autoimmune and immune mediated disorders (e.g. autoimmune thrombocytopenia) and of microbial diseases respectively. At present, the range of potential applications has broadened largely because it is now possible to administer much larger doses both of the Ig and of specific antibody. Recently, two developments have revolutionised the field of Ig therapy: i) the development of IgG products, obtained from plasma fractionation, safe for i.v. administration in high doses and ii) the production of monoclonal antibodies of mouse and human origin. Although i.v. Ig are safe from transmission of HBV and HIV, a number of preparations have transmitted non-A, non-B hepatitis.

Hence, the manufacture of a consistently safe i.v. Ig is a major issue for plasma fractionators.

Monoclonal antibodies offer great potential advantages for the treatment of infection; antibodies can be selected in vitro for maximum protective activity and those with highest potency can be produced. Clinical trials of some monoclonal antibodies such as hepatitis B Ig have already started. Although anti-Rh D Ig has been produced in several laboratories by EBV-transformed lymphoblastoid cell lines, we do not know yet whether such Igs will be effective in suppressing the immune response to the Rh antigen and consequently whether they will eventually replace polyclonal anti-Rh in the prophylaxis of Rh haemolytic disease of the newborn. Safety aspects that need to be addressed before monoclonal antibodies are used to treat humans include the risks of sensitization to foreign protein and the possibility of contamination with viral material (e.g. EBV) from the antibody-producing cell line. Since many hybridomas are derived from malignant cells, proof of lack of carcinogenicity in the final product will be required.

A novel approach is the production of «humanised» monoclonal antibodies containing variable regions of mouse origin attached to human constant regions. The aim of this approach is to minimize immunogenicity of the Ig while preserving high specificity and high concentration.

Three separate gene loci, each containing variable and constant chain genes, comprise the Ig gene system. The three chain genes are located on three separate chromosomes and DNA rearrangement plays an essential role in Ig diversity. In view of this complexity and high degree of specificity of Igs, it is very unlikely that recombinant DNA technology will ever be applied to the production of Igs and hybridoma technology will continue to be the most reasonable approach.

Financial considerations

Expenditure on blood and blood products by each country is climbing at a rate higher than inflaction. For example, it has been predicted by an international market research firm, that in 1991 the USA will spend US $2.2 billion in blood derivatives compared with $1.4 billion in 1986. The main reason for this high increase is AIDS; HIV antibody screening tests, methods of inactivation of viruses in blood products and the difficulties in recruiting donors due to the irrational fear of contracting AIDS by donating blood. Fractionated plasma products which used to account for only 10 % of the sales of blood derivatives, are outpacing blood components (red cells, platelets and plasma) in sales growth.

At present, plasma derivatives made through genetic engineering procedures are more expensive than human plasma and it is difficult to predict what the expenditure in blood products will be in 10-20 years time. In addition, the volume of plasma protein product required per patient and its therapeutic efficacy, the yield of the particular protein per kg of raw plasma, the availability, the relative production cost, the purity (i.e. lack of foreing, non-human protein) and the ease of viral inactivation, will determine which product will be prepared by rDNA technology and which will continue to be made by methods of plasma fractionation. As stated above, although production of albumin by rDNA is possible, at present it is far from being cost effective.

Considerations of the introduction of a new procedure or blood product must include, in addition to its therapeutic advantages, the cost of its use. This could become a critical factor in the next few years. For example, if FVIII is produced by rDNA technology, but albumin is not (tonnes are needed as opposed to approx. 500-800 g of FVIII), the cost of plasma-derived albumin will rise to cover a considerable proportion of the costs of fractionation. But, since many clinicians are overprescribing and misusing albumin, if its cost becomes prohibitive, the global use of albumin is bound to decline significantly. At present, the information available it's too scarce and it is too early to state with any certainty what the consequences of new technologies will be in the field of blood transfusion. It is clear that evolution has occurred and many careful considerations will need to be given to the introduction of novel practices in blood transfusion.

FURTHER READING

Quality Control Guidelines for blood transfusion services 1986. Council of Europe – Strasbourg.

Wagstaff W. Quality assurance of blood products. Progress in Transfusion Medicine 2. JD Cash (Ed), Churchill Livingstone 1987.

World Health Organization 1981. The collection, fractionation, quality control and uses of blood and blood products. WHO, Geneva 1981.

Raine AEG. Hypertension, blood viscosity and cardiovascular morbidity in renal failure: implications of erythropoietin therapy. The Lancet 1988; **i:** 97-99.

Royston D, Bidstrup BP, Taylor KM, Sapsford RN. Effect of approtinin on need for blood transfusion after repeat open-heart surgery. Lancet 1987; **ii:** 1289-1291.

Bidstrup BP, Royston D, Taylor KM, Sapsford RN. Effect of aprotinin on need for blood transfusion in patients with septic endocarditis having open-heart surgery (letter). Lancet 1988; **(i):** 366-367.

Kickler TS, Spivak JL. Effect of repeated whole blood donations on serum immunoreactive erythropoietin levels in autologous donors. Am Med Assoc 1988; **260:** 65-67.

NIH. Perioperative Red Cell Transfusion Consensus Development Conference, June 27-29, 1988. Nat Inst of Health, Bethesda, Maryland, USA.

Czer L, Bateman T, Gray R, et al. Prospective trial of DDAVP in treatment of severe platelet dysfunction and hemorrhage after cardiopulmonary bypass (Abstract). Circulation 1985; **72,** suppl. 3, section III, 130.

Salzman EW et al. Treatment with desmopressin acetate to reduce blood loss after cardiac surgery. N Engl J Med 1986; **314:** 1402-1406.

Mollison PL, Engelfriet CP, Contreras M. Blood Transfusion in Clinical Medicines. Blackwell Scientific Publications 1987, Oxford.

Kahn RA, Allen RW, Baldassare J. Alternate sources and substitutes for therapeutic blood components. Blood 1985; **66:** 1-12.

Gould SA, Rosen AL, Sehgal LR et al. Fluosol-DA as a red cell substitute in acute anaemia. N Eng J Med 1986; **314:** 1654-1656.

Rouger P, Goossens D, Karaouby Y, Salmon C. Therapeutic human monoclonal antibodies: from the laboratory to clinical trials. Trends-Biotechnol 1987; **5:** 217-219.

Van Der Plas J, De Vries Van Rossen A, Damm HB, Bakker JC. Preparation and physical characteristics of a hemoglobin solution modified by coupling to 2-nor-2-formylpyridoxal 5'-phosphate. Transfusion 1987; **27:** 425-430.

NIH Consensus Conference. Perioperative Blood Transfusion. JAMA 1988; **260:** 2700-2703.

DHSS. Health Notice: Procurement – Product Liabity. Health Publications Unit 1988, Heywood.

Dept of the Trade and Industry. Guide to the Consumer Protection Act 1987 – Product Liability and Safety Provisions. Consumer Safety Unit – Dept of Trade and Industry 1987, 10-18 Victoria St. London SW1.

Slichter SJ, Deeg HJ, Kennedy MS. Prevention of platelet alloimmunisation in dogs with systemic cyclosporine and by U-V irradiation or cyclosporine loading of donor platelets. Blood 1987; **69:** 414.

Adams GA, Swenson SD, Rock G. Survival and recovery of human platelets stored for 5 days in non-plasma medium. Blood 1987; **67:** 672.

Burrows L, Tartter PI. Effect of blood transfusion on colonic malignancy recurrence rate. Lancet 1982; **ii:** 662.

Moffat LEF, Sunderland GT. Relation between recurrence of cancer and blood transfusion. Br Med J 1985; **291:** 971.

Blumberg SA et al. Relation between recurrence of cancer of the colon and blood transfusion. Br Med J 1985; **290:** 1037-1039.

Francis DM, Judson RT. Blood transfusion and recurrence of cancer of the colon and rectum. Br J Surg 1987; **74:** 26-30.

Parrott NR et al. Effect of perioperative blood transfusion on recurrence of colorectal cancer. Br J Surg 1986; **73:** 970-973.

Ross WB. Blood transfusion and colorectal cancer. J Roy Coll Surg Ed 1987; **32:** 197-201.

Sehgal LR, Rosen AL, Gould SA. Haemoglobin solutions as red cell susbstitutes. Prog Transf Med 1988; **3:** 128-144.

Djordjevich L, Miller IF. Synthetic erythrocytes from lipid encapsulated hemoglobin. Exp Hematol 1980; 584-592.

Hunt CA, Burnett RR, MacGregor RD, Strubbe AE. Synthesis and evaluation of a prototypal artificial red cell. Science 1985; 1165-1168.

Mayoral J, Djordjevich L, Miller IF, Ivankovich AD. Evaluation of synthetic erythrocytes as blood substitutes. ACEMB 1985.

National Institutes of Health. Fresh Frozen plasma. Indications and risks. J Am Med Assoc 1985; **253:** 551-553.

Nienhuis AW. Hematopoietic growth factors. New Engl J Med 1988; **318:** 916-918.

Galvani D, Griffiths SD, Cawley JC. Interferon for treatment, the dust settles. Br Med J 1988; **296:** 1554-1556.

Farmer MC, Gaber BP. Encapsulation of hemoglobin in phospholipid: Surrogate red cells in vitro and in vivo. Biophys J 1984; **45:** 201a.

Wood WI et al. Expression of active human factor VIII from recombinant DNA clones. Nature (London) 1984; **312:** 330-337.

Lawn RM, Adelman J, Bock SC et al. The sequence of human albumin serum cDNA and its expression in *E. coli.* Nucleic Acids Res 1981; **22:** 6103-6114.

Barker L. World blood resources: The relationship of medical needs for whole blood, red cells, and plasma derivatives to blood collection, plasmapheresis and fractionation practises. Report for the World Blood Resources Subcommittee of the Council of the International Society of Blood Transfusion, Montreal, Canada, August 1980.

Gitschier J, Wood WI, Goralka TM et al. Characterization of the human Factor VIII gene. Nature 1984; **312:** 326-330.

Toole JJ, Knopf JL, Wozney JM et al. Molecular cloning of a cDNA encoding human antihaemophilic factor. Nature 1984; **312:** 342-347.

Anson DS, Austen DE, Brownlee GG. Expression of active human clotting Factor IX from recombinant DNA clones in mammalian cells. Nature 1985; **315:** 683-685.

De la Salle H, Altenburger W, Elkaim R, Dott K, Dieterle A, Drillien R. Active gamma-carboxylated human Factor IX expressed using recombinant DNA techniques. Nature 1985; **316:** 260-270.

Edwards CA, Piet MPJ, Chin S, Horowitz B. Tri (n-butyl) phosphate/detergent treatment of licensed therapeutic and experimental blood derivatives. Vox Sanguinis 1987; **52:** 53-59.

Clark AJ et al. Pharmaceuticals from transgenic livestock. Tib Tech 1987; **5:** 20-24.

Sehgal LR, Rosen AL, Gould SA et al. Haemoglobin solutions as red cell substitutes. In Progress in Transfusion Medicine 1988; vol 3, 128-144. Ed JD Cash.