Immunohaematopoietic stem cell transplantation in South Africa

- The first 40 years – An experimental and clinical model for approaching restorative medicine

Abstract

Hippocratic teaching enshrines literally centuries of ethical principles in medicine. The foremost of these being to heal and do no harm – an ideal for which all clinicians strive but advances are seldom – if ever – entirely risk free. The way in which such balance is achieved presupposes our remaining perpetual students seeking to couple current knowledge with a clear perspective of perceived benefit versus defined hazard. Wisdom to this degree is found in few individuals but, nevertheless, as an ideal, serves to define the true scientist who, tenaciously, uncompromisingly and over prolonged periods systematically completes basic research for subsequent translation into clinically relevant practice.

Nowhere are these concepts better illustrated than in the burgeoning field of stem cell biology. Media sensationalism, commercial or monetary opportunism and self-aggrandisement continue to confuse the vital task of advancing this aspect of medicine but doing so within moral and religious constraints. A further impediment to progress is failure to distinguish between embryonic and adult options. In the former the steps closely replicate physiology and the totipotential of the inner cell mass is the basis for the parallels in reproductive and therapeutic cloning. Intrinsic to these techniques must be definitions as to when life begins and debates to extend all the way to the American Congress! The possibilities are enormous but require safeguards to be generated from dispassionate debate primarily between scientists and ethicists rather than politicians. These issues are not our immediate concern.

Rather, in contrast, starting material from already formed organs has restricted capacity for differentiation and proliferation with a different challenge to resolve – can one source give rise to another function? To grasp this
Introduction
The emerging role of stem cells in biology continues to accelerate thereby focusing attention on terminology. Two broad categories of donor material exist. Their fundamental behaviour is widely divergent and continues to generate debate between scientists, philosophers, ethicists and, increasingly but a lot less helpful, politicians!

HUMAN EMBRYONIC ORIGIN is considered here only to clarify fundamental features. The defining property is a capacity to produce all the tissues that make up a complete individual where the first step is fusion of ovum and sperm to generate the zygote. During passage down the fallopian tube and early phases of implantation in the uterine wall the morula gives rise to blastocyst in which recognisable inner cell mass has pluripotentiality but lacks the capacity to form extra-embryonic structures. During gastrulation three germ layers form known as the ectoderm, endoderm and mesoderm and these constitute the blueprint for a complete offspring.

It follows that capacity to recapitulate physiology \textit{ex vivo} or in cultures is momentous. In scientific terms recovery of the blastocyst and implantation into a surrogate uterus is feasible as seen in meticulously selected cases generally at fertility clinics. A variation is reproductive cloning where, through the process of somatic cell nuclear transfer, the original or haploid nucleus from the oocyte is removed and replaced with a mature counterpart where the chromosomes originate from the patient. The therapeutic equivalent is an interesting variation where the early blastocystic stage is cultured \textit{in vitro} with differentiation being driven towards multipotentiality and exemplified by muscle, neurones or blood depending on modulating influences in the medium. \textit{(Figure 1)}

These somewhat different options have profound moral and religious overtones encompassing criteria for when life commences and predicate the need for inclusive and sensitive monitoring at every stage to avoid even the slightest abuse.

\textbf{Figure 1: Contrasting stem cell methodologies}

Schematic to highlight the cardinal difference based on the source of starting population with stem cell derived either from embryo or adult tissue.

\begin{itemize}
\item \textbf{Physiology:} Oocyte + sperm \rightarrow zygote \rightarrow blastocyst \rightarrow uterine implantation \rightarrow Normal offspring
\item \textbf{Reproductive cloning:} Enucleated oocyte + nuclear transfer \rightarrow transfer \rightarrow uterine transfer \rightarrow blastocyst
\item \textbf{Therapeutic cloning:} Enucleated oocyte + nuclear transfer \rightarrow harvested embryonic stem cell
\item \textbf{Adult cell cloning:} Haematopoietic stem cell \rightarrow expand \textit{ex vivo} \rightarrow seed to new environment \rightarrow Plastic response to altered phenotype
\end{itemize}

Indeed the recent South Korean claims highlight the sensitivity of this area. Enormous restraint, transparency and responsible reporting are mandatory for constructive and sensible regulation but this needs to be by knowledgeable peers working in the field if future benefit is not to be denied.

ADULT SELF RENEWING populations provide the counterpart and are found in all organs where they provide for natural repair as in wound healing or sustaining blood formation from the bone marrow. The latter has been studied extensively and is the basis for autologous or allogeneic immunohaematopoietic grafting. Long years of intensive study have established that numbers with repopulating potential are relatively small but, can be mobilised into the circulation for recovery: however keep in mind their protracted exposure to environmental toxins and the accumulation of genetic injury over the lifetime of the donor. Despite such drawbacks preliminary data support the possibility that they can be expanded ex vivo and, furthermore, manipulation of culture conditions subtly alter the phenotypes. If correct, the tantalising concept arises in which such multipotential progenitors actually have less circumscription that long espoused as conventional dogma but rather that anatomical boundaries can be transgressed. For example incorporation of nominally blood forming precursors into heart muscle after infarction or, cartilage, ligaments and even nerves, aiding recovery, may be possible. Whether such plasticity exists at all or, in the diametrically opposed viewpoint, is a reality proposes the existence of a living reagent with wide ranging restorative properties. Not surprisingly there is support for intensive study to meticulously separate fact from fantasy. This approach is somewhat less emotion-laden than the use of pre-implantation material but, is nevertheless, every bit as relevant and, accordingly, a foundation must be established in soundly based, rigorous tested and confirmed science. Unless restraint is exercised there is a risk of generating premature and unjustified expectations which will tarnish all efforts by reputable investigators to proceed constructively. This charge has been given national priority by convening a study group under the aegis of Stellenbosch University jointly with the South African Medical Research Council. Here one group will seek to consolidate experience that has accumulated with international collaboration over the last 35 years and continue to explore the enigmatic haemangioblast, or even earlier forms, in restorative medicine. The events leading to the current status are therefore summarised as a record as to how this point was reached.

Bone marrow transplantation as a model

EXPERIMENTAL HAEMATOLOGY, using a murine system, demonstrated reappearance of blood formation in a medullary cavity rendered aplastic by lethal irradiation and showed that this was not due to plasma factors but cell mediated. Elegant studies continue to define fundamental aspects of homing to special sites known as niches, where particular environmental influences dictate differentiation in what is then described as the haematopoietic inductive microenvironment. Localisation of precursors in relationship to marrow trabeculae is constant and dictated by a complex system of interactions controlled by stromal ligands in the form of adhesion molecules or cytokines recognising cognate receptors on primitive progenitors. Complimentary observations that a unique relationship exists between supporting mesenchyme and early committed lineage find support in the culture system described by Professor Michael Dexter. Particularly relevant is an in vivo splenic assay where the content of the growing colonies vary according to the particular site in this organ where they eventually develop. In broadest context these interactions have stimulated developments widely tested including the use of higher primates. It is not unreasonable to anticipate that adult progenitors have an intrinsic capacity to undergo phenotypic remodelling depending on how they are stimulated and, in this way, participate in repair of muscle, ligaments and perhaps even neurones. It might be asked, somewhat rhetorically, if quiescent populations exist in solid organs, presumably dedicated to just this task, why are extrinsic sources needed? This, and other inconsistencies, await systematic study.

CLINICAL STUDIES were pioneered by Professor E Donnall Thomas and generations of his research fellows drawn from every corner of the globe. Although a number of additional groups were at about the same time involved in developing these procedures it would be invidious not to recognise the contributions of Professor George Santos at Johns Hopkins, and the outstanding radiobiologists such as Dirk van Bekkum, in the Netherlands.
Each of these, and others of considerable talent and stature, have pooled resources to gradually advance understanding and improve outcome. Nevertheless, given the monumental contributions that largely started in Seattle and led to the establishment of the Fred Hutchinson Cancer Centre with trainees heading up units in practically every country, it is fitting and appropriate to acknowledge the ongoing leadership of this long time friend, colleague and a most deserving Nobel laureate.

Development of the Cape Town programme

The seed is sown

A number of sequential steps characterised first the introduction and then the systemic consolidation of the infrastructure which preceded the first bone marrow allograft in South Africa at Groote Schuur Hospital in 1972. 1964 saw the first attempted procedure in this country. A 23 year old male medical student with severe aplasia received a somewhat arbitrary volume of anticoagulated aspirate from a brother. Engraftment did not occur and he died as a result of neutropenic septicaemia. In retrospect this is not surprising since little was known about histocompatibility and even less about graft composition or immunosuppression. This event, combined with an ever-expanding literature on related topics including human leukocyte antigens system, reports of success in leukaemia and Fanconi or aplastic anaemia made an indelible impression during fellowship years spent with Professor Clement A Finch in Seattle where there was close contact with the multidisciplinary program directed by Dr Thomas.

Inbred rabbit strains were used rather than mice

On return to this country it was a considerable honour to be appointed as Foundation Professor of Haematology at the University of Cape Town. Fortuitously, just at this time, Professor Christiana Neethling Barnard had carried out the first successful human heart transplant and ignited interest from Professor Eugene B Dowdle and Dr M C Botha in providing immunologic assays for the histocompatibility testing. Not surprisingly many were caught up in this new field and the fledgling department started funnelling all resources and energy into marrow grafting as the major direction for the future. From the first day encouragement and support was abundantly available from faculty, staff and colleagues making it possible, somewhat painstakingly at times, to create the infrastructure culminating in the first successful such procedure within two years. This was not a random event but rather the starting point of a carefully planned and projected long-term core activity.

Methodology in evolution

Laboratory processing was set up by collaboration with Professor Bruno Speck and Dr Alois Gratwohl in Basel in which, rather than using mice, inbred rabbit strains were selected to refine the details of each step. Harvesting femoral marrow, as a surgically sterile procedure under general anaesthetic, and the preparation of a monocellular suspensions was perfected using a series of stainless steel screens of decreasing pore size. After radiotherapy of recipients, skillfully provided by Dr Basil Shepstone and Professor Rossall Sealy, autografting was used to compare intravenous infusion to block re-implantation but showed no difference. This required establishment of a vivarium and the considerable skills of Mr Graham Manual as a cardinal member of the team are remembered with appreciation. Graft monitoring was primitive using only mononuclear numbers and all efforts to antigenically match pairs was unsuccessful. The alternative measurement, shifted to clonogenic assays and started in the Heath-Robinson constructed carbon dioxide incubator built by Dr Joan Parker. Rapidly erythroid, granulocyte and eventually mixed colony growth was standardised as the reference point for quality control and it was possible to demonstrate a linear relationship between these measurements and engraftment. The scene was set to translate laboratory results to patients.

The clinical programme

Heart breaking frustration occurred in a number of areas during this first decade. The need to interact with more experienced colleagues in other parts of the world was impeded by working in a country blanketed by an intense academic boycott directed at the apartheid policy. Despite this a steady stream of investigators from most of the major centres found ways of visiting and, reciprocally hosted ourselves in initiating and consolidating this activity. Benefit undoubtedly accrued from the local cardiac and renal successes.
Much of the early hazard attributable to neutropenia, compounded by unavoidable immunosuppression, created substantial morbidity and mortality. Here one continues to reflect on the extraordinary degree of encouragement from the surgeons including access to their specialised beds and nursing expertise in the eleventh block. The challenge of overcoming potentially or sometimes lethal complications reinforced the need to draw on expertise scattered throughout the School. This led to creation of the multidisciplinary Haem Team which, to this day, routinely includes pulmonary, cardiac, renal and infectious disease consultants in the moment-to-moment management of these complex clinical problems.

A series of rather distinct, albeit overlapping periods, can be recognised in this historical perspective:

Apheresis technology provided a means to discontinue the supply of blood and single unit platelets in glass bottles! Replacement of this rather quaint but obsolete service was made possible by recognising the superiority of the cell separator and the IBM 2990 was secured by donation. Over the ensuing years this methodology became standard and gradually permeated the commercial transfusion services first in the Western Cape and subsequently in other provinces. This period was stimulated by a visit from Professor Jeane Porter Hester and this association continues actively today. (Figure 2) A major benefit was the ability to recover the corresponding population having engraftment potential from the peripheral blood which became the norm and further refinement is a current priority.

A further innovation was the creation of a dedicated platelet donor panel made up of doctors and staff in the department and unselfishly from volunteers throughout the Groote Schuur Hospital at every level from gardeners through janitorial staff to senior consultants. This philosophy underscored the approval of the new activity and serves as a reminder of the esprit de corps found in the corridors of the old hospital.

Unfractionated marrow was used initially requiring conventional immunosuppression with corticosteroids and methotrexate. (Figure 3) Rejection and acute as well as chronic graft-versus-host disease paralleled world experience and, while as elsewhere some success was achieved, the morbidity and the mortality of these complications exacted a heavy toll on nursing and medical staff alike. At this point the team became aware of the need to add a staff psychiatrist, a dedicated social worker and physiotherapist, all innovations remaining central to the current programme more than 30 years later.

Alternative sources include umbilical cord blood which is available primarily on a dedicated basis within families. Access to this product is further coordinated through the South African Bone Marrow Donor Registry and our membership to Eurocord. This is a valuable resource and may well be a further productive area for study in non-haematopoietic mediated tissue repair.

Whether, in the South African context, there is a place for banking remains far from reality although a local working party was established some years ago to explore this possibility: (Jacobs and Wood unpublished).
Unrelated donors may be needed where siblings are not available. To overcome this obstacle we formed the South African Bone Marrow Donor Registry jointly with Dr Arthur Bird as Medical Director of the Western Province Blood Transfusion Service and Professor Ernette du Toit as Head of the Provincial Laboratory for Tissue Immunology. Scope continues to expand and, as the Hub centre there are links with the corresponding European Organisations and the American National Donor Program where the Constantiaberg team is designated as a transplant and harvest centre.6,15 There has gradually been increasing recognition of these activities that found expression in the 6th International Donor Registry Conference from 26th May – 27th May 2006 in Cape Town.

Cyclosporin A became available as a result of cooperation studies with Professor Jean Borel in Sandoz in Switzerland. Years of a fruitful collaboration explored the role of this unique undecapeptide in the postgraft period but the anticipation of complications disappearing was unfortunately short lived. Undoubtedly incidence and severity of these immunologically related phenomena decreased but they remained a major challenge to the investigators.16 Monoclonal antibodies became available as a result of a still-active association with Professor Herman Waldmann and Dr Geoff Hale first in Cambridge subsequently in Oxford. Here, as part of the Campath Users Group, there was an opportunity to investigate the \textit{ex vivo} use of these immunoglobulins for T lymphocyte depletion. A previously untested modification, namely their admixture to the harvest in-the-bag without any further manipulation,17 was described and subsequently, having been tested in a number of other collaborating centres, emerged as effective, thereby avoiding the need for any further immunosuppression. Interestingly this is now a standard form of management which, at least in our hands, is associated with a high remission rate at least in acute myeloid leukaemia. Acute and chronic graft-versus-host disease, in traditional sense, are no longer seen. However an interesting forme fruste of these unusual immunologic manifestations occasionally occurs in the form of a skin rash which is regarded as a late onset of the acute variant. A further association is with cytomegaloviral seroconversion but progression to pneumonitis or other organ involvement has largely been prevented by proactive ganciclovir administration.

Audit, accreditation and current status
For an unbroken period of 30 years the same group have reported consecutive cases first to the International and then to the Autologous registries which are now combined as Center for International Blood and Marrow Transplant Research. Latterly accountability is also scrutinised for continued active participating membership of the American National Donor Program. Such peer review is regarded as mandatory to maintain standards and, following audit, accreditation has been uninterrupted for the past three decades.
In 1995 this designated team, complete with affiliations, relocated to a newly built transplant facility in a private academic centre. Results are constantly analysed in adults: (P Jacobs, L Wood, J Haveman, J Juritz - unpublished) (Figure 4) and children: (L Wood, J Juritz, J Lund, P Jacobs - unpublished) (Figure 5). In recent years activity has increased placing a strain on the staff. This has been relieved by introducing a fellowship program that will have particular focus on research projects including the investigation of immunologic reconstitution, the pattern and consequences of infections and evaluation of reduced intensity conditioning regimens. The importance of formal academic association is seen by designation as a satellite within the Division of Clinical Haematology in the Department of Internal Medicine - Stellenbosch University – Tygerberg Academic Hospital.

In approximately 1980 these procedures were also started by Professor Thomas H Bothwell in the Department of Medicine, University of Witwatersrand under the leadership of Professor Werner Bezwoda and continue with Professor Paul Ruff. Here there are some parallels to the University of Cape Town where the original programme continues at the Groote Schuur Hospital with private referrals catered for separately. A number of other facilities are in different stages of development including Johannesburg, Pretoria, Bloemfontein and Durban.

Extrapolation to restore medicine rationale

Philosophers pose questions in abstract form. Ethicists and moralists refine these concepts by debate leaving scientists to provide answers through systematic study. This framework is helpful in understanding how changes in emphasis from the conventional treatments over yesteryear come to be replaced by more proactive or preventive current strategies. Thus, as an example, none would question the use of prostheses to improve the mechanical disability when limbs are lost or the increasing sophistication that makes these more user friendly, the use of living tissues is, however, viewed quite differently. Here, although the distinction is somewhat artificial, future advances lie in two broad directions. Firstly there is the controversial area of embryonic tissue and its use in reproductive or therapeutic cloning. It could responsibly be argued that this field needs to advance but to do so in a properly regulated way thereby capitalising on the immense potential benefit in a wide range of congenital and acquired disorders that affect the human race.

Legitimate reservations extend to how the material is derived and there is a clear need to regulate clinical application. These concerns are not central to the present debate. Secondly, contrastingly and of major relevance, as the population ages with subtle changes in immunologic competence and decreasing tissue integrity there is a need for a conceptual shift to supplement physiological replacement where examples include degenerating neural tissue as in Parkinson’s and Alzheimer’s diseases, diabetes, damaged joints and cardiomyocytes. It is in this context that the immunohaematopoietic stem cell can be examined further looking at the question of plasticity and some preliminary data of its role in selected organ systems.

Plasticity

Careful scrutiny of experiments in nature, exemplified by response to injury, reveals the presence of rests or cellular collections that have the capability of expanding into mature and functional progeny. These are more abundant where turnover is rapid as in the epithelium and blood. Such a belief may be too restrictive since evidence is accumulating that differentiation may occur across lineage boundaries and this phenomenon, described as plasticity, may offer a novel therapeutic strategy to facilitate tissue regeneration.
Interpretation of publications need to be tempered by an understanding of technical details that include selection of the study material and the distinction between fusion as opposed to transdifferentiation. Nevertheless the ability to harvest readily accessible adult stem cells may open previously inconceivable treatment options. In view of the ease with which bone marrow can be obtained and evidence of its flexibility in generating myeloid and lymphoid lineages as well as mesenchyme – the latter capable of differentiating into bone, cartilage and fat highlight the choice of haematopoietic stem cells to examine advances in this field.

**Current or potential applications**

Understanding the way in which the latter population gives rise to blood formation interchangeably with non-haematopoietic lineages challenges the concept of the hierarchical model and has led to speculation that the facts are more compatible with the kinetic concept in which there is a continuum within this compartment. Importantly these emerging concepts sound a word of warning since neoplastic changes may occur in the course of these manipulations.

Two extensive reviews argue the evidence for the presence of organ specific stem cells and possible contributions from marrow stem cells. In reviewing data from mice coupled with limited clinical experience, and recognising the need, as noted above, to separate fusion from transdifferentiation, a number of areas emerge for specific study and where there is already data available. Prominent among these are skeletal and cardiac muscle, liver, skin, gastrointestinal tract, lung, pancreas, kidney and of particular interest, the central nervous system.

**A concluding perspective**

Conventional bone marrow transplantation has, in the last 40 or 50 years, undergone enormous development and this continues to be translated into great saving of life. Morbidity is being reduced as support and nutritional intervention improve, infectious episodes are more effectively treated and graft-versus-host disease skillfully controlled. Immunohaematopoietic stem cells are routinely recovered from the circulation after mobilisation using apheresis techniques and there is daily wider recourse to umbilical cord blood. In parallel histocompatibility matching is more precise and the role of reduced intensity conditioning extends the age of the recipient and offers an opportunity to test potentially new immunologically mediated benefits exemplified by anti-tumour destruction through supplementary donor lymphocyte infusion. Such momentous advances provide an enormous international database from which to start moving these sophisticated procedures innovatively into the field of restorative medicine. Thus, given that most organs house small populations of stem cells with specific capacity to effect local repair, and nowhere is this better demonstrated than in blood formation, there arises the question of whether such rather narrow concepts should not be reviewed. Leaving aside the more controversial issues of reproductive and therapeutic cloning from embryos and focusing on the adult population with regenerative capacity, the answer would appear to be – yes. Evidence increasingly supports observations that this population has a property described as plasticity to explain their presence during reparative processes in a wide range of many other sites from muscle, through skin and gastrointestinal tract to the central nervous system.

Questions about the interpretation of this phenomenon abound including debates as to whether this is simply cell fusion or, in actual fact, transdifferentiation. Nevertheless it is precisely these important considerations that led to the formation of a South African Study Group that recently held an inaugural workshop to coordinate and support locally based investigators. Understandably one of the discrete working parties, and for good reason, will focus on the long experience of bone marrow transplantation in this country, aiming to develop it as a model to continue the exploration of the potentially important role of these procedures in this new field of restorative medicine.

**Acknowledgements**


Christine Dölling helped with the bibliography review and Sharon Smith excellently typed the manuscript. Our thanks to both research assistants.
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