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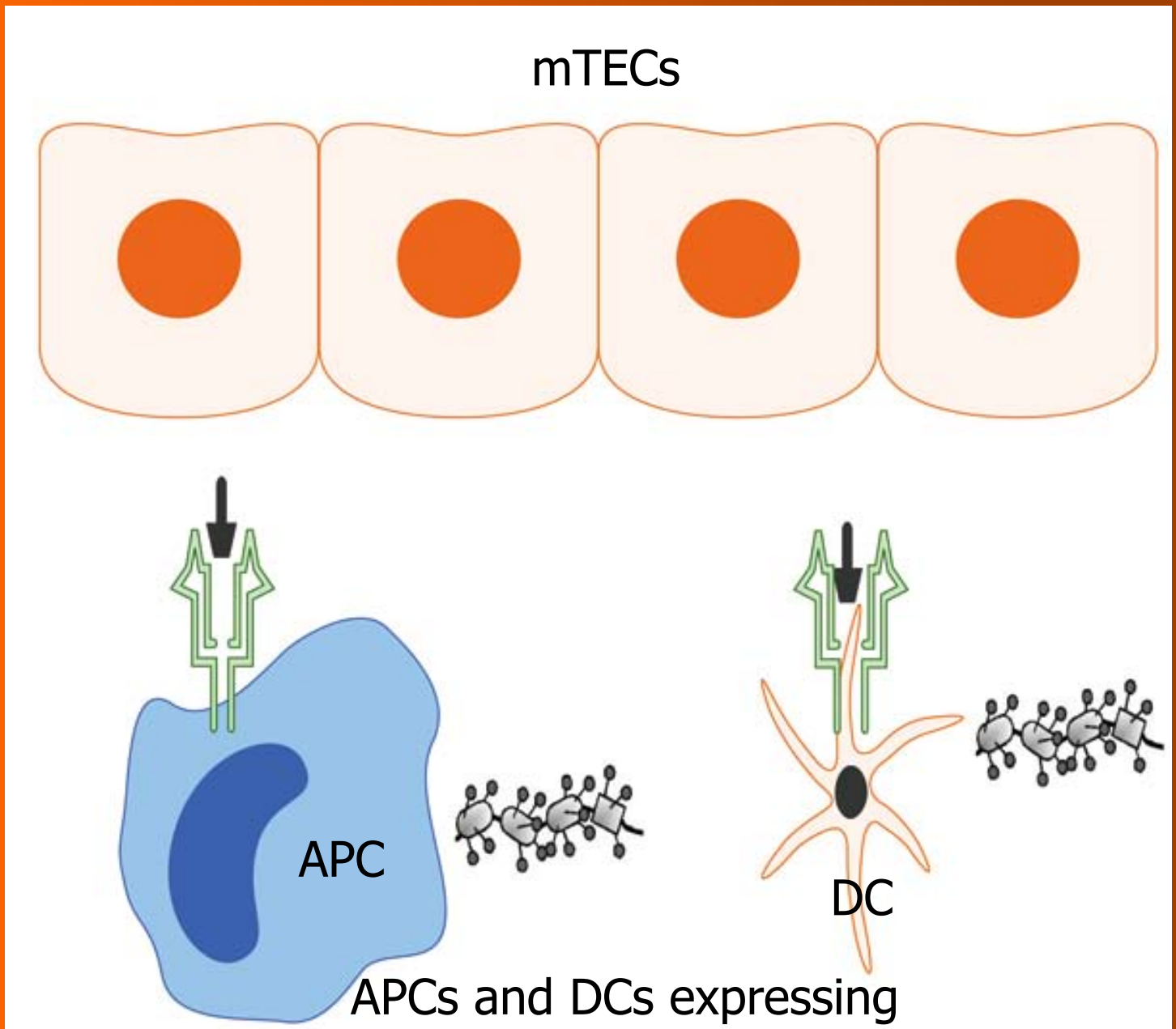

APCs and DCs expressing differentiated CD45 isoforms and presenting antigen to mTECs

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WJSC covers topics concerning all aspects of stem cells: embryonic, neural, hematopoietic, mesenchymal, tissue-specific, and cancer stem cells; the stem cell niche, stem cell genomics and proteomics, and stem cell techniques and their application in clinical trials.

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FRONTIER

In utero transplantation: Disparate ramifications

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Abstract

In utero stem cell transplantation, which promises treatment for a host of genetic disorders early in gestation before disease effect stems from Ray Owen's seminal observation that self-tolerance, is acquired during gestation. To date, in utero transplantation (IUT) has proved useful in characterizing the hematopoietic stem cell. Recent observations support its use as an in vivo method to further understanding of self-tolerance. Preclinical development continues for its application as a treatment for childhood hematolymphoid diseases. In addition, IUT may offer therapeutic options in the treatment of diabetes among other diseases. Thus IUT serves as a technique or system important in both a basic and applied format. This review summarizes these findings.

Key words: Self non-self discrimination; Immune ontogeny; Stem cell transplantation

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INTRODUCTION

A variety of genetic disorders of the hematolymphoid system have been treated successfully by transplanting hematopoietic stem cell (HSC) into pediatric and adult patients. However, this procedure's applicability is limited by compatible donor availability, the use of immunosuppression/cytoablation and the potential development of graft-*vs*-host disease (GVHD). Further, for some pediatric diseases (*i.e.*, the glycogen storage diseases, thalassemia, *etc.*) significant disease progression begins during fetal development such that even at birth the patient is irreversibly compromised. In these conditions, postnatal bone marrow transplantation can ameliorate disease progression but is unable to correct the existing damage[1].

Successful allogeneic HSC transplantation is accomplished by donor reconstitution of hematopoiesis (usually after depletion of defective or undesirable host cells) following the infusion of donor HSC in anticipation of their homing to appropriate hematopoietic tissues. This requires overcoming the immune barrier of the host, limiting the ability of donor immune cells to mediate GVHD and facilitation of homing and engraftment of donor HSC where the inductive milieu will promote regulated reconstitution of hematopoiesis. In the clinical setting many of the complications of postnatal HSC transplantation are the result of procedures such as ionizing radiation and chemotherapy used to limit host immune rejection or deplete host marrow to allow adequate donor engraftment/expression.

In theory, intrauterine stem cell transplantation (IUSCT) presents significant advantages in comparison to postnatal stem cell (SC) transplantation, which if optimized, could present less toxic treatment protocols for many genetic/developmental diseases. Advances in molec-

ular biological techniques and improvements in obstetric procedures such as chorionic villous sampling permit the collection of fetal material to diagnose these conditions early enough in gestation to allow the use of IUSCT for treatment prior to the onset of irreversible organ damage. This enthusiasm is founded on the concept that establishment of graft tolerance without cytoablation and without GVHD can be accomplished in a cost effective manner. Unfortunately, at present, the only clinical applications for which IUSCT has proven successful are in diseases associated with host immunodeficiency[2,3].

As we reported in our earlier review, intrauterine transplantation (IUT) as a method emanated from attempts in the first half of the 20th century to understand self-tolerance and accept the concept of autoimmunity[1,4]. While these observations ruled out genetic determinism and identified fetal developmental events as responsible, it became clear in the latter half of the last century lead by the experiments of Le Douarin in birds, that how cellular deletion and cellular suppression (the main mechanisms underlying self-tolerance formation and maintenance) were formed during development were and are poorly understood[5]. Indeed, only recently have we identified the importance of and probably of more interest, why gestational timing is crucial to utilizing IUT both clinically and experimentally[6].

Nonetheless, once the concept that introduction of foreign antigens (*i.e.*, HSCs) could be introduced into the fetus and persist, clinicians were encouraged that this breakthrough might offer a path to cure. Supporting evidence that IUSCT could work comes from the observations on naturally occurring chimerism in dizygotic twins in both large animals and humans. Here, the mixing of HSC through the placental circulation throughout gestation may result in enhanced sibling cell expression (> 30%)[7]. Natural chimerism is common[8]. In some veterinary diseases amelioration of disease phenotype has been attributed to sibling cell expression[9-11]. Experimental work transplanting a variety of HSC into large and small animal fetuses was supportive[2].

Clinical experience has been disappointing. In part this may be due to inadequate understanding of immune ontogeny and the importance of time in rendering tolerance. As often, procedures were performed after our proposed gestational tolerance window (approximately weeks 12-16 gestation) closes. However there are well-documented examples of proper timing and administration of an adequate SC dosage to treat a disease that theoretically should benefit for which there is no engraftment or if engrafted no clinically meaningful expression is seen[12,13]. What we hope to show is given recent experimental observations; a successful protocol for treatment of even non-immunodeficiency diseases is within our grasp.

In this report, we would like to expand both experimentalists' and clinicians' appreciation of the power of this procedure (ramifications). Our observations on immune ontogeny reveal IUT's utility in unlocking events fundamental to understanding self-tolerance. Also, we believe it possible that this fetal tolerance phase in a large animal may allow *in vivo* investigation of biologic systems that may permit the development of alternative solutions to a number of clinical problems. Herein, we review its experimental background, propose the antigen exposure model to explain developmental acquisition of self-tolerance, examine impediments and promising areas for optimizing the procedure for treatment of childhood diseases and finally speculate regarding this technique's utility as *in vivo* platform for therapeutics.

BACKGROUND

The discovery of common placental circulation between dizygotic twins as the explanation for the freemartin coupled with the development of erythrocyte antigen profiling in cattle allowed Ray Owen to determine that dizygotic twins were chimeric with their sibling's blood cells after birth. Thus, he concluded that self-tolerance is acquired during fetal development and not innate[1,14]. Subsequent experiments in mice, sheep and cattle confirm that imprinting (Rapid time-dependent irreversible behavioral learning that occurs during development originally described by Karl Lorenz in young geese.) during fetal development is responsible for immune tolerance in adult life[15-17]. In direct experiments, fetal transplantation with allogeneic HSC, xenogeneic HSC or RTV (Gene expression following retroviral vector transfer in utero follows similar kinetics to that observed after cellular transplantation. We believe this is due to the establishment of recipient transplantation tolerance to the gene product hence we use transplantation rather than transfer.) in sheep reveals a gestational window of receptivity to engraftment mirroring the acquisition of self-tolerance[6,18]. The SC xenografts are highly expandable and are associated with extensive differentiation. Indeed, besides normal hematopoietic lineages, differentiated cardiac, gastrointestinal, liver and pancreatic islet cell activity can be demonstrated years after transplantation[19,20]. This window occurs in mice later in gestation but successful long-term engraftment and expression of both allogeneic and xenogeneic HSC has been realized[1,21-23]. For example, in Figure 1 we note bi-lineage human chimerism in a mouse following IUT at the proper gestational age; significant expression required graft stimulation with human growth factors (see below). In summary, self non-self discrimination is relative and time dependent.

We have found performing allogeneic and xenogeneic IUT in sheep (a large animal) useful in the study of the HSC[24,25]. Formal study using timed gestational sheep identified the developmental event permissive for long-term engraftment receptivity as the period immediately following thymic demarcation (The timing of thymic demarcation into cortex and medulla varies with the size of the animal. In mice demarcation occurs at 66%, sheep 35% and humans 31% expiration of gestation. It is thought that the medulla is primarily responsible for deletional and cellular tolerance.). This phase is finite last-

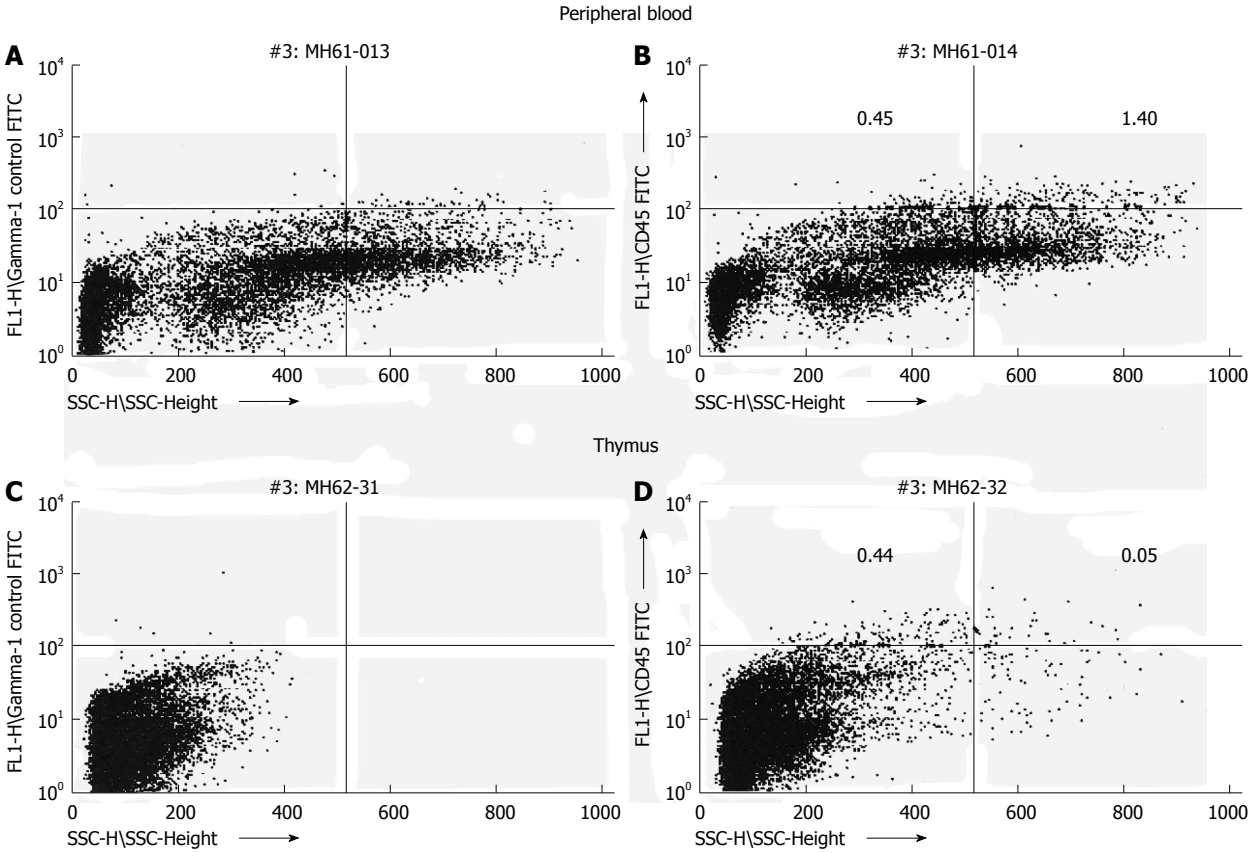

Figure 1 Transplantation of human hematopoietic stem cell (CD34+) during the murine engraftment window results in bi-lineage expression and relevant cell migration of the differentiated human progeny 6 mo after transplantation. Human CD45 cells exhibit different side scatter characteristics (low side scatter: lymphocytes; high side scatter: granulocytes) dependent on organ tested (A, C: Control mouse; B, D: Experimental mouse)[23].

ing no more than 30 d in sheep (term gestation 145 d) or 2 d in mice (term gestation 21 d). The ability to determine exactly when in gestation engraftment receptivity occurs permitted parallel experiments on lymphocyte ontogeny in sheep. These experiments identified the thymus as the site of immune activity (CD45 differentiation) during the transplant receptivity window. CD45 isoform differentiation occurring only in the thymus included all identifiable lineages: T cell, B cell and antigen presenting cell (APC). Unfortunately, ovine natural killer (NK) cell specific reagents were not available to track NK cell development. Evidence for thymic deletion of T and B cells is seen. It is important to note that these observations suggest B cell tolerogenesis does not occur in spleen, bone marrow or Peyer's patches[6,26-30]. Studies using retroviral vector (RTV) transplantation in sheep demonstrate preferential thymic epithelial cell (TEC) expression of the gene product during this period with the development of specific lymphocyte unresponsiveness to the gene product and inability to generate viral specific antibody after birth[31,32]. Thus, B cell deletion (perhaps mediated by the developing "deletional TEC matrix") is likely formed in the thymus during the window. Further, expression of the RTV gene product in the TECs in Hassall's corpuscles (the proposed site Treg formation) and our phenotype experiments during this period (JSP, unpublished observations) suggest that, the onset of Treg formation occurs simultaneous with or near the conclusion of the tolerance window[31]. Reports by McCune and colleagues support this conclusion, as Tregs in humans are a predominant T cell phenotype after thymic demarcation. They further propose that fetal HSC may provide additional stimulus to this thymic differentiation cascade[33,34].

RAMIFICATIONS: DISSECTION OF SELF-TOLERANCE

These observations support a model for fetal tolerance induction where antigens (either autologous, allogeneic or xenogeneic) in the extracellular compartment of the developing thymus during this temporal phase are processed by the differentiating TEC progenitors and APC component of the thymic stroma and presented to developing immunocompetent cells (identifiable by changes in CD45 isoform expression) to form the tolerance repertoire. This two-stage antigen exposure model is presented in Figure 2. Presentation of surface antigens on cells intercalating the developing thymus during this finite period might also contribute. Endogenous gene derived presentation (to explain solid organ tolerance) is not consistent with our observations and unresponsiveness in the NK lineage must also be acquired during this period[35-42]. The extracellular antigen exposure

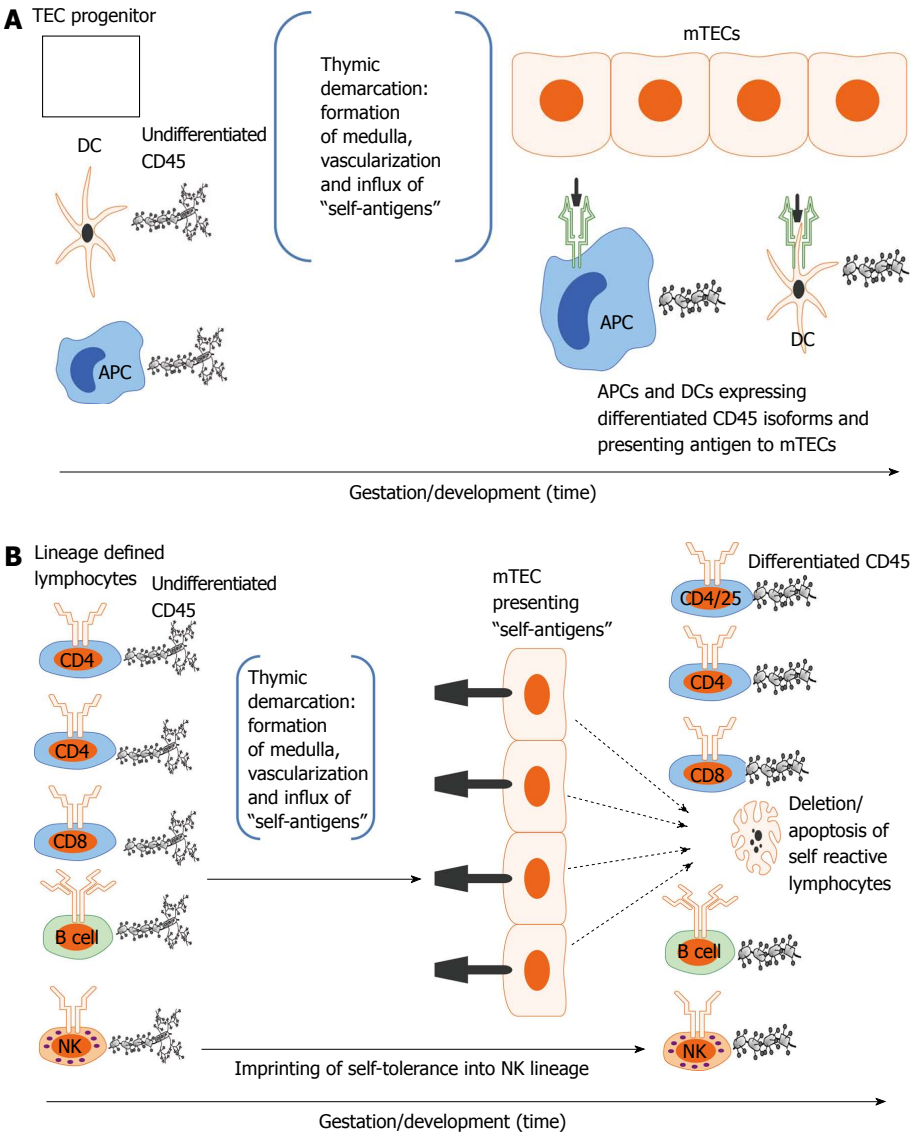

Figure 2 Developmental acquisition of self-tolerance: Antigen exposure model. A: Thymic epithelial (TEC) progenitors mature in intimate contact with dendritic cells (DCs) and likely other antigen presenting cells (APCs) thus forming the thymic medulla (*i.e.*, thymic demarcation). With vascularization, self-antigens (*i.e.*, antigens present during the finite tolerance window) from the periphery are processed and presented to medullary thymic epithelial cells (mTECs). The developing mTEC matrix is only receptive to antigen presentation during the tolerance window (time dependence) and subsequently exhibits on its surface the self-antigen repertoire (see 3. Ramifications: Dissection of immune tolerance); B: All lymphoid compartments [T, B and natural killer (NK) cell] enter the thymus expressing undifferentiated CD45 but are lineage defined. They then migrate through the thymus encountering mTECs expressing the self-antigen repertoire. CD4, CD8 and B cells reactive to self-antigens are deleted. CD4/CD25 cells reactive to self-antigens are rendered suppressive (Treg) in Hassal's corpuscles. NK cells are imprinted in an undefined fashion (likely based on major histocompatibility complex exposure) to become tolerant to self. All differentiated lymphocytes (*i.e.*, cells that have undergone CD45 isoform maturation and are not self-reactive or are tolerogenic (*i.e.*,Treg) then migrate to the spleen or other secondary lymphoid organs (see 3. Ramifications: Dissection of self-tolerance)[6,21,31-37,42].

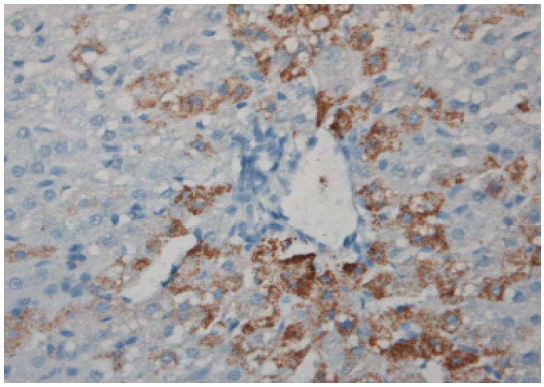

Figure 3 Induction of sheep tolerance to human hepatocytes by transplantation of human hematopoietic stem cells during the temporal engraftment window (note absence of sheep round cell infiltration). Sheep liver stained for human specific antibody 11 mo following transplantation[49].

model would explain the absence of rejection of both hematolymphoid and cellular components of solid organ xenografts and the marked expandability of grafts following transplantation[43,44]. It suggests that the NK cell compartment undergoes thymic imprinting during this critical phase to explain the absence of NK cell mediated rejection. Evidence supporting thymic processing and adaptability of NK cells is reported[42,45-47]. It seems unlikely that Treg(s) could account for the complete absence of NK cell mediated rejection (Figure 3)[48,49]. Thus these observations demonstrate the value of IUT as a method to explore mechanisms underlying induction of self non-self discrimination.

RAMIFICATIONS: TREATMENT OF CHILDHOOD HEMATOLYMPHOID DISEASES

Xenotransplantation of human HSC following IUT in sheep and mice results in human cell engraftment and differentiation. In sheep this includes cardiac, liver, intestinal and pancreatic cells. Differentiated cellular function manifested by identification of circulating human proteins in transplanted sheep and mice is presented in Table 1[23,49-52]. The absence of rapid circulatory clearance

Table 1 Human proteins detected in circulation of animals transplanted in utero with human stem cells

Animal transplanted	Human protein detected
Sheep	IgM[1]
Sheep	Albumin[47,48]
Sheep	Factor Ⅷ[1]
Sheep	C-peptide[49,50]
Sheep	α-fetoprotein[1]
Mouse	IgM[23]

[1]Unpublished observation by Esmail D Zanjani. IgM: Immunoglobulin M.

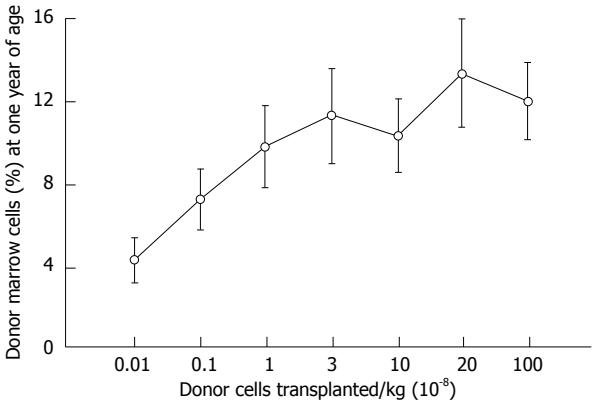

Figure 4 Levels of allogeneic engraftment plateau with transplantation of log-fold increases in donor cells in sheep. This is most consistent with a model of saturation kinetics with engraftment limited by available receptive sites. An identical curve is seen in the xenogeneic human-sheep model[2].

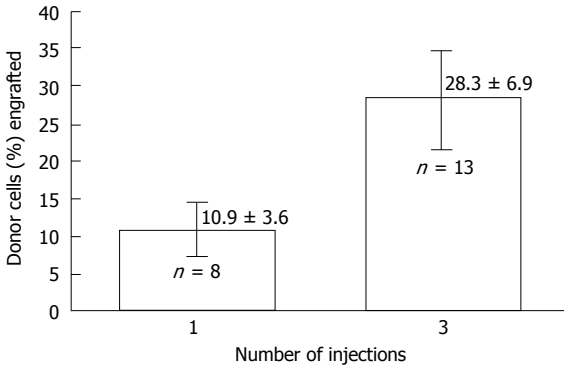

Figure 5 The effect of dividing cell dose on engraftment in sheep. Transplantation of the same total number of cells as a single injection *vs* a series of three injections given 1 wk apart is shown. The engraftment with divided doses is significantly higher supporting the concept that engraftment is limited at any particular time by the number of available receptive sites[2].

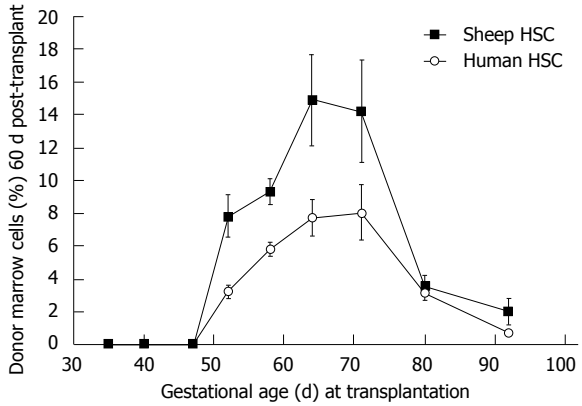

Figure 6 Allo- or xenogeneic stem cell bone marrow engraftment in sheep following transplantation during the temporal window when self non-self discrimination occurs (note parallel kinetics). Engraftment receptivity is gestational age-dependent[6].

speaks to the profound ability of IUT to alter the self non-self paradigm. IUSCT is thought to provide a number of therapeutic advantages over postnatal transplantation besides tolerance including expanding bone marrow space (or niche), sterile environment, proliferative environment and preempting clinical disease. Diseases thought amenable to IUT include hemoglobinopathies, immunodeficiency states and inborn errors of metabolism leading to storage diseases (mucopolysaccharidosis and mucolipidosis)[1].

As Figures 4 and 5 reveal maximizing cell dosage and serial cell administrations can improve donor engraftment yet, there remain a set of problems in achieving adequate donor proliferation to ameliorate and/or cure these diseases[2]. The parameters proposed that may be limiting successful clinical application include: (1) failure to induce tolerance and/or an inadequate engraftment frequency (chimeric incidence); and (2) limited graft expression: gestational developmental impediments, maternal derived inhibition, lack of selective advantage (*i.e.*, inadequate donor proliferation).

Tolerance/engraftment following IUT

Both natural and experimentally derived hematopoietic chimeras demonstrate immune tolerance using skin graft acceptance and mixed lymphocyte reactions. Deletional and cellular tolerance has been reported[16,43,53-55]. Marrow engraftment levels 60 d after HSC transplantation in sheep using xenogeneic or allogeneic HSC reveals a parallel kinetic profile with a peak level when transplantation is performed between days 65-70 gestation (Figure 6). This suggests accurate gestational timing may be critical to accessing the full panoply of immune tolerance mechanisms (*i.e.*, deletion, regulatory cell formation and anergy among all immune competent lineages) forming during the window[56]. Transplantation before the onset of the window and maternal derived histoincompatibility may limit engraftment or expression but in general tolerance is achieved[6,54,55,57-60]. The short tolerance induction period in mice may contribute to occasional microchimerism and NK cell mediated rejection[21,57,58]. In sheep and mice, engraftment and/or expression can be augmented long after birth (*i.e.*, further supporting the achievement of long-term immune tolerance and memory). In Table 2, groups of 6 sheep were assessed for tolerance to allogeneic HSC and then re-transplanted after birth with same donor HSC resulting in marked improvement in the level of engraftment. Flake has demonstrated similar findings

Table 2 Groups of 6 sheep were assessed for tolerance to allogeneic human stem cells and then re-transplanted after birth with same donor human stem cells

Allogeneic sheep HSC render recipient sheep[1] tolerant following in utero transplantation		
Stimulator	Responder	Stimulation index[2]
Donor	Donor	0
Recipient	Recipient	0
Donor	Recipient	0-8
Recipient	Donor	58 ± 11
Pooled	Donor	69 ± 12
Pooled	Recipient	78 ± 12
Postnatal infusion of allogeneic same donor HSC augments engraftment in tolerant sheep[3]		
% donor cells at birth	*n*	% increase[4]
6-10	4	86 ± 29
11-15	5	63 ± 22
> 15	4	21 ± 11

[1]Representative sample of 6 chimeric lambs; [2]Variation of mixed lymphocyte reaction (MLR) previously reported[32]; [3]Tolerance determined *via* MLR[32]; [4]Assessed 6 mo after postnatal stem cell infusion (3×10^8 cells/kg) in thirteen chimeric lambs rendered tolerant. HSC: Human stem cell.

in the allogeneic mouse model[43]. In a similar vein, mixed lymphocyte reaction unresponsiveness has been detected following xenogeneic in utero transplantation (unpublished observations).

As noted above, if performed during the proper phase in gestation, the SC engraftment incidence is high in both large and small animal models. Mouse models are hampered by the short induction period and multiple small fetuses to inject resulting in a diminished engraftment frequency. In large animals, the engraftment frequency is considerably higher. For example, less than exhaustive examination of the pancreas in sheep revealed a chimeric incidence of 79%[51].

Limited graft expression following IUT

Gestational developmental impediments: The course of hematopoietic ontogeny is associated with an orderly and predictable switch in primary sites of hematopoiesis. Mammalian hematopoietic sites are gestational age dependent starting in the yolk sac migrating to liver and spleen then finally lodging in the bone marrow. Yet, the marrow contributes little to circulating peripheral blood until just prior to birth. The mechanisms underlying this migration to marrow reflect bone and marrow ontogeny including formation of the osteoblastic niche. With regard to transplanted HSC, we reported a similar engraftment gradient dependent on bone and marrow maturity with little peripheral blood expression[61-63]. This osteoblastic niche (site of primitive HSC engraftment) matures by day 65 and may in part influence the peak in marrow engraftment seen following transplantation (Figures 6 and 7). But again, the liver/spleen remains the main site of hematopoiesis until just prior to birth. These represent serious homing and maturational impediments to achieving therapeutic levels of donor cell expression to treat diseases that are clinically evident during gestation.

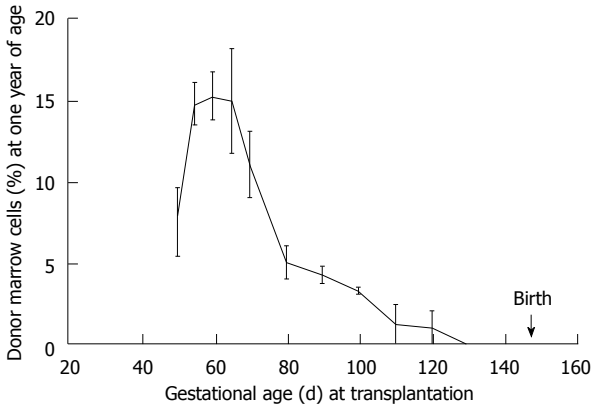

Figure 7 Maximal donor cell engraftment in sheep occurs during the mid-to-later stages of the tolerance induction period. This period follows thymic demarcation and formation of the osteoblastic niche. Further, engraftment is durable as the engraftment kinetics are similar to that noted at 60 d post transplant supporting our contention that the graft is viewed by the recipient as self (see Figure 6)[2].

Maternal-derived inhibition: Maternal derived inhibition either through histoincompatible maternal lymphocyte microchimerism or maternal alloantibodies may limit engraftment/expression. To address this issue, we observed that maternal histocompatible donor grafts from matched cord blood improved peripheral blood donor expression (2.75% *vs* 0.93%) in comparison to relatively mismatched grafts[62].

Inadequate competitive advantage to promote donor expression: Allogeneic SCs can repair genetic hematologic defects due allograft responsiveness to endogenous growth factors[22,64-66]. Donor-species-specific growth factors improve graft expression in xenografts confirming establishment of tolerance and importance of donor stimulation in either an allogeneic or xenogeneic environment following IUT[1,44]. A promising approach that needs further examination is the use of autologous stroma. In our hands, co-transplantation improved graft expression (donor hemoglobin) during gestation suggesting this may be a useful tack to treat diseases overtly manifest during gestation (Figure 8)[67].

The end of the tolerance induction phase in large animals is followed by exponential growth[6]. Thus, augmentation of donor engraftment/expression with transplantation of high dose donor SC and/or autologous stroma during this exponential growth phase to compete with endogenous/recipient SC differentiation and expansion should prove fruitful. Late retransplantation would mimic the mixing of hematopoietic cells in natural chimera that occurs through the placental circulation during and after closure of the gestational tolerance window and can result in the enhanced expression of sibling cells. The sheep is the maternal/fetal system most stable to test this hypothesis[68]. Studies in mice support this notion as endogenous marrow ablation after birth with retransplantation of donor HSC can essentially replace recipient cells[69].

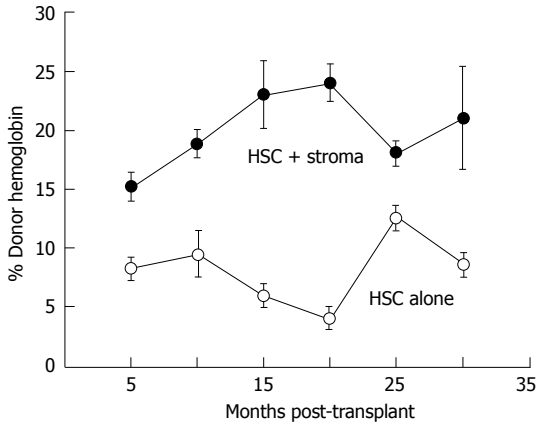

Figure 8 Co-transplantation with autologous adult sheep stromal cells improves allogeneic donor hematopoietic stem cell expression. Donor expression (circulating hemoglobin) persists more that 30 mo after transplantation. Donor hemoglobin levels in the peripheral blood of sheep co-transplanted with autologous adult bone marrow stroma (n = 4) are significantly higher (P < 0.01) than those transplanted with adult T-depleted bone marrow mononuclear cells alone (n = 3)[67]. HSC: Human stem cell.

Clinical experience and roadmap for implementation

Therapeutic success following IUT in fetuses diagnosed with a variety of genetic diseases has been disappointing. In repair of the severe combined immune deficiency (SCID) defect, the transplanted cells have a competitive advantage and a poorly populated functional organ in which differentiation may occur[3]. Yet, there are well-documented examples of proper timing and administration of an adequate SC dosage to treat a disease that theoretically should benefit for which limited graft expression is seen[12,13]. Moving forward will require a more coordinated approach, a clinical trial in an ideal candidate disease.

This coordinated approach should identify a candidate disease that will provide "proof principle" that our theories are correct in a clinical setting. While at first glance one might conjecture that transplantation in X-linked SCID was successful because the fetus is immunodeficient, an alternative and more likely explanation is that an under populated developing thymus provided important stimulus for expansion and expression of the donor graft (a proliferative stimulus similar to Mintz's demonstration in anemic mice)[22]. For this reason thalasemia is a poor disease with which to test the feasibility of IUT. Here there is often marked over growth of endogenous marrow cells limiting donor cell engraftment during development[1]. In a similar fashion, failure to observe enhanced expression in mismatched allogeneic grafts in sheep suggest that either maternal derived inhibition limits donor expression or autologous stromal factors are required to augment graft expression. Likely mechanisms would be through either stromal major histocompatability complex matching to preferentially nurture the developing autologous SC or autocrine or paracrine factors which specifically stimulate autologous SC. Thus histocompatability is an important factor not due to potential recipient immune inhibition (*i.e.*, failure of tolerance induction) but by providing a graft that is not prone to maternal immune inhibition or providing (as yet undefined) autologous factors supporting graft expression (evidenced by our report demonstrating improved peripheral blood expression during development in allogeneic grafts co-transplanted with autologous stroma, Figure 8)[67].

In summary, IUT recapitulates normal acquisition of self-tolerance. Experimentally, tolerance and engraftment are readily achieved. The problem remains identifying a disease that will promote graft expansion, limit maternal interference or selectively promote the graft through autologous stimulation. This likely requires retransplantation either late in gestation or shortly after birth (during exponential growth) to provide a dosing advantage to allow adequate numbers of donor HSC to compete with recipient HSC to ameliorate disease in this tolerant environment. Risks such as procedural, the possible need for multiple IUT procedures, infection and GVHD likely are not prohibitive[2]. Since the majority of the genetic disorders correctible with HSCT can be diagnosed early in gestation to allow for IUHSCT, it is important that attempts be made to correct these deficiencies before birth. Use of compatible donors, determining the optimal gestational age and co-transplantation of donor-derived stroma/mesenchymal SCs may help achieve this end.

Ramifications: In vivo platform for therapeutics

The formation of differentiated human cells/organs (without evidence for donor/recipient cell fusion) and functional human proteins following transplantation allows speculation as to the feasibility of IUT in applied therapeutics in an autologous or allogeneic setting. This would necessitate resolving problems such as cell/protein separation and isolation, as well as contamination with and transmission of infectious agents. Yet, development of effective methods to mitigate these impediments could unlock uncharted therapeutic possibilities. The finding of differentiated human functional proteins and evidence that engraftment/expression can be expanded before or after birth offers the possibility that IUT in a large animal could provide a source of autologous human proteins. In a similar fashion, effective cell isolation (hematopoietic cells seem the most feasible) might offer the possibility of autologous cell transfusion for clinical support.

While the above possible applications of IUT may be better served using *ex vivo* bioreactors or systems, *in vivo* use of sheep or an alternative animal of similar size to humans may be useful with regard to development of organs for transplantation. Our laboratory has been investigating this with regard to the liver and endocrine pancreas[49-52]. Our studies involving the endocrine pancreas have demonstrated long-term circulating human insulin following SC transplantation. The ease with which islets can be isolated for transplantation makes this an ideal organ to determine feasibility of IUT as a therapeutic modality for human organ transplantation. For both systems, the critical determinant will be the role of a single SC (or as Owen described them embryonal ancestral cells) in or-

gan repair[1,70].

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BRIEF ARTICLE

Comparison of phenotypic markers and neural differentiation potential of multipotent adult progenitor cells and mesenchymal stem cells

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Abstract

AIM: To compare the phenotypic and neural differentiation potential of human bone marrow derived multipotent adult progenitor cells (MAPC) and mesenchymal stem cells (MSC).

METHODS: Cultures of MAPC and MSC were established in parallel from same samples of human bone marrow (n = 5). Both stem cell types were evaluated for expression of pluripotency markers including Oct-4 and Nanog by immunocytochemistry and reverse-transcription polymerase chain reaction (RT-PCR) and expression of standard mesenchymal markers including CD14, CD34, CD44, CD45, CD73, CD90, CD105 and human leukocyte antigen (HLA)-ABC by flow cytometry. After treatment with neural induction medium both MAPC and MSC were evaluated for expression of neural proteins [neuronal filament-200 (NF-200) and glial fibrillar acidic protein (GFAP)] by immunocytochemistry and Western blotting and neural genes [NF-200, GFAP, Tau, microtubule-associated protein (MAP)-1B, MAP-2, neuron-specific enolase (NSE) and oligodendrocyte-1 (Olig-1)] by quantitative real-time-PCR.

RESULTS: MAPC had small trigonal shaped while MSC had elongated spindle-shaped morphology. The MAPC expressed Oct-4 and Nanog both at gene and protein levels, whereas MSC were negative for these pluripotent markers. MAPC were negative for HLA-ABC while MSC had high expression of HLA-ABC. In addition, MAPC as compared to MSC had significantly lower expression of CD44 (36.56% ± 1.92% *vs* 98.23% ± 0.51%), CD73 (15.11% ± 2.24% *vs* 98.53% ± 2.22%) and CD105 (13.81% ± 3.82% *vs* 95.12% ± 5.65%) (P < 0.001, for all) MAPC cultures compared to MSC cultures treated with neural induction medium had significantly higher fold change expression of NF-200 (0.64), GFAP (0.52), Tau (0.59), MAP-2 (0.72), Olig-1 (0.18) and NSE (0.29) proteins (P < 0.01 for Olig-1 and P < 0.001 for rest) as well as higher fold change expression of genes of *NF-200* (1.34), *GFAP* (1.12), *Tau* (1.08), *MAP-1B* (0.92), *MAP-2* (1.14) and *NSE* (0.4) (P < 0.001 for all).

CONCLUSION: MAPC can be differentially characterized from MSC as Oct-4 and Nanog positive stem cells with no expression of HLA-ABC and low expression of mesenchymal markers CD44, CD73 and CD105 and when compared to MSC they possess greater predilection for differentiation into neuro-ectodermal lineage.

Key words: Bone marrow; Human multipotent adult progenitor cells; Human mesenchymal; Stem cells; Pheno-

typic markers; Neural differentiation

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INTRODUCTION

Multipotent adult progenitor cells (MAPC) and mesenchymal stem cells (MSC) are two predominant non-hematopoietic stem cell types of the bone marrow stroma, that have enormous therapeutic properties including regenerative therapy for neurodegenerative disorders[1,2]. The MAPC are pluripotent stem cells with capacity to differentiate into cells of all the three germ layers[3-5]. They have been variously described in literature as multipotent adult stem cells (MASC)[6], mesodermal/multipotent progenitor cells (MPC)[7], marrow-isolated multi-lineage inducible cells[8], adult pluripotent stem cells[9] and embryonic like stem cells (ELSC)[10] by different groups. The expression of pluripotent and neural markers in MAPC and their increased mobilization in patients with neurodegenerative diseases like stroke show potential role of these stem cells in neurogenesis[11-13]. The MSC are mesodermal progenitors that are committed to differentiate into cells of mesodermal lineage[14]. However, some studies have shown that in addition to their mesodermal commitment they also differentiate into cells of neuro-ectodermal lineage, claiming their role in neurogenesis[15,16]. However, it is not known whether MAPC or MSC possess superior neurogenic potential and very less information is available on phenotypic differences between MAPC from MSC so it is also difficult to distinguish them from each other.

Therefore, the aim of the present study was to evaluate the expression of pluripotency and mesenchymal markers and to carry out a parallel comparison of neural differentiation potential of MAPC and MSC derived from the same samples of human bone marrow.

MATERIALS AND METHODS

Isolation, culture and characterization of MAPC and MSC

Subjects included in the study (n = 5) consisted of 2 healthy donors for bone marrow transplant patients and 3 patients with suspected iron deficiency anemia where bone marrow (BM) was done to look for iron stores, who otherwise had a normal BM. After informed consent, 5mL of BM aspirate was collected from each individual for this study, and it was divided into two equal parts for growing MAPC and MSC from the same sample in parallel.

The MAPC were cultured using Verfaillie's protocol[3]. Briefly, bone marrow mononuclear cells (BMNC) of the marrow aspirates were depleted of CD45 and GlyA positive cells were cultured in growth medium consisting of 53.8% 1.5 mg/mL bovine serum albumin (BSA) mixed Dulbecco's modified Eagle's medium (DMEM)-low glucose medium (Gibco, www.invitrogen.com), 40% MCDB-201 (Sigma, www.sigmaaldrich.com), 2% fetal bovine serum (FBS) (Hyclone, www.thermoscientific.com), 1% ITS+1 Supplement (Sigma), 0.5 μmol/L dexamethasone (Sigma), 0.1 mmol/L L-ascorbic acid (Sigma), 1% LA-BSA (Sigma), 1% penicillin/streptomycin (Gibco), 10 ng/mL each of platelet-derived growth factor-BB (R and D, www.rndsystems.com) and epidermal growth factor (R and D) under hypoxic condition. The sub-confluent cultures were trypsinized and further expanded under same culture conditions to get optimal number of cells. The MAPC were characterized by expression of Pluripotency markers Oct-4 and Nanog and their differentiation into cells of three germ layers viz. neuronal (ectodermal cells), endothelial (mesodermal cells) and hepatocytes (endodermal cells).

The culture of MSC was carried out using Prockop's protocol[17]. Briefly, BMNC were cultured in complete medium consisting 88% of α-MEM Medium, 10% of FBS, 2 mmol/L of L-Glutamine and 100 units/mL of penstrep (all from Gibco) under normoxic condition. The MSC were characterized by expression of conventional mesenchymal markers and their differentiation into mesodermal cell including bone and fat cells.

Flow-cytometry

The phenotypes of MAPC and MSC were analyzed by two color flow cytometry at 3rd passage using human leukocyte antigen (HLA)-ABC [fluorescein isothiocyanate (FITC)]/CD44 [phycoerythrin (PE)], CD34 (FITC)/CD73 (PE), CD14 (FITC)/CD105 (PE) and CD45 (FITC)/CD90 (PE) (all from Serotec, www.abdserotec.com). The flow-cytometer used was FACS-calibur (Becton Dickinson) and data analysis was done using FACS express software.

Reverse-transcription polymerase chain reaction

Expression of *Oct-4* and *Nanog* genes was done by reverse-transcription polymerase chain reaction (RT-PCR). Total RNA of the cells was extracted using RNeasy mini RNA isolation kit (Invitrogen). Two μg of total RNA was reverse transcribed into cDNA using random hexamers (Invitrogen). The cDNA was normalized by amplification of β-actin, The PCR conditions included denaturation at 94 ℃ for 4 min, amplification cycles 35 and elongation temperature 72 ℃ for 30 s with annealing. The amplicons were resolved on 2% agarose gel (Sigma-Aldrich) and pictures acquired using gel documentation system (Alpha Imager, www.proteinsimple.com).

Immunocytochemistry

The expression of pluripotency genes *Oct-4* and *Nanog* on MAPC and MSC was analyzed by immunocytochemistry. The cells were fixed with 4% para-formaldehyde (Sigma Aldrich) in PBS for 1 h at room temperature. The fixed cells were incubated overnight at 4 ℃ with following

Table 1 Sequence of primers used in reverse-transcription polymerase chain reaction and real time-polymerase chain reaction

No.	Primer	Sequence	Accession number
1	4-Oct	f: 5′-CGTGAAGCTGGAGAAGGAGAAGCTG-3′ r: 5′-CAAGGGCCGCAGCTTACACATGTTC-3′	NM_002701.4
2	Nanog	f: 5′-GCCGAAGAATAGCAATGGTGTG-3' r: 5'-CCAGGACTGGATGTTCTGGGTC-3'	NM_024865.2
3	NF-200	f: 5′-CAGAGCTGGAGGCACTGAA-3′ r: 5′-CATCTCCCACTTGGTGTTCC-3′	NM_021076.3
4	GFAP	f: 5′-GAGTACCAGGACCTGCTCAA-3′ r: 5′-TTCACCACGATGTTCCTCTT-3′	NM_002055.4
5	MAP-1B	f: 5′-GCGGAGACAGTACCTTCGGAG-3′ r: 5′-CCGACGACCACCAGCAAGTAG-3′	NM_005909.3
6	MAP-2	f: 5′-TCAGAGCCAATTCGCAGAG-3′ r: 5′-TGTTGTC TGTTGATCCGATTTT-3′	NM_002374.3
7	Tau	f: 5′-TCATTAGGCAACATCCATCATA-3′ r: 5′-CACCTCGTCAGCTAGCGT-3′	NM_001203252.1
8	NSE	f: 5′-TCTGCAGTCCCGAGATCCCAGC-3′ r: 5′-CTGATGAGGGCTGGCGCGAT-3′	NM_001975.2
9	Olig-1	f: 5′-GCCCCACCAAGTACCTGTCTC-3′ r: 5′-GGGACCAGATGCGGGAAC-3′	NM_138983.2
10	β-actin	f: 5'-GCTCGTCGTCGACAACGGCTC-3′ r: 5'-CAAACATGATCTGGGTCATCTTCTC-3'	NM_001101.3
11	GAPDH	f: 5'-GATTTGGTCGTATTGGG-3′ r: 5'-TCCACGACGTACTCAGC-3'	NM_002046.3

NF-200: Neuronal filament-200; GFAP: Glial fibrillar acidic protein; Olig-1: Oligodendrocyte-1; NSE: Neuron-specific enolase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; MAP-1B: Microtubule-associated protein-1B; MAP-2: Microtubule-associated protein-2.

primary antibodies: Nanog and OCT-4 (1:200 dilution) (Chemicon, www.millipore.com) After washing with PBS, cells were incubated with 1:500 diluted goat anti-mouse immunoglobulin G (IgG) (Fab)$_2$ FITC (Abcam, www.abcam.com) as secondary antibody and stained with Hoechst dye. The pictures were acquired by fluorescent microscope (Nikon 80i, Japan).

Differentiation into neuro-ectodermal cells

We used following protocol for differentiation of MAPC and MSC into neuronal cells. The cells were plated into 12-well plates at a density of 2000-2500 cells/cm^2 and incubated in neuro-ectodermal induction medium consisting of 98% basal medium (57% DMEM low glucose, 40% FBS, 1% Pen-strip, 1% ITS+1, 0.1 mmol/L L-ascorbic acid, 0.5 μmol/L dexamethasone), 100 ng/mL basic fibroblast growth factor (R and D systems), 100 ng/mL Noggin (R and D systems), 20 ng/mL NT-3 (R and D systems), 10 ng/mL brain-derived neurotrophic factor (R and D systems), 10 ng/mL glial cell line-derived neurotrophic factor (R and D systems), 20 μmol/L RA (Sigma), 1X B-27 supplement (Gibco), 1X 2-ME (Gibco). The differentiated cells were characterized as neuronal cells by immunocytochemical detection of neuronal filament-200 (NF-200), microtubule-associated protein 2 (MAP-2) and glial fibrillary acidic protein (GFAP) on the cells using MAP-2 (Abcam), NF-200 and GFAP primary antibodies (Biovision). Goat anti-mouse IgG (Fab)$_2$ FITC (Abcam) as secondary antibody and Hoechst dye staining as described above.

Western blotting

Western blotting was done using primary antibody NF-200 (200 kDa), GFAP (51-52 kDa) (Biovision), Tau (52 kDa), MAP-2 (280 kDa), Olig-1 (28 kDa) and neuron-specific enolase (NSE) (47 kDa) (Abcam) and β-actin (42 kDa) (Abcam) and horseradish peroxidase conjugated corresponding secondary antibodies. The signals were detected using an enhanced chemiluminescence detection system (Amersham Biosciences. www.gelifesciences.com).

Real-time PCR

The quantification of neuronal gene expression in MAPC and MSC was carried out by real time PCR. Total mRNA was isolated from the undifferentiated and neuro-ectodermal differentiated cells following single step mRNA isolation method using RNA isolation kit (Invitrogen). Total mRNA (2 μg) was reverse transcribed to cDNA using RT-PCR kit (Applied Biosystems) following manufacturer's instructions. Real time analysis for NF-200, GFAP, MAP-2, MAP-1B, Tau, oligodendrocyte-1 (Olig-1), NSE and normalizing gene glyceraldehyde 3-phosphate dehydrogenase (Table 1) was performed using Sybr Green Master mix as per the manufacturer's instruction (Applied Biosytems), analysis were done on Light-cycler 480 (Roche) and fold changes in gene expression was calculated using $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The results were calculated as mean ± SE. The statistical significance between MAPC and MSC comparisons was determined by using one-way analysis of variance test. P values < 0.05 were considered to be statistically significant.

RESULTS

Morphology and phenotypes

The MAPC and MSC both grew as adherent cells in culture but they were morphologically distinct from each other. The MAPC had small trigonal morphology while the MSC were large cells having elongated spindle shaped morphology (Figure 1A).

The MAPC had no expression of HLA-ABC (0%) while MSC had high expression of HLA-ABC (93.32% ± 2.58%). The MAPC compared to MSC had significantly lower expression of CD44 (36.56% ± 1.92 % *vs* 98.23% ± 0.51%), CD73 (15.11% ± 2.24% *vs* 98.53% ± 2.22%), CD105 (13.81% ± 3.82% *vs* 95.12% ± 5.65%) ($P < 0.001$ for all). Both MAPC and MSC had high expression of CD90 (99.80% ± 0.14% *vs* 99.47% ± 0.44%; $P > 0.5$) and no expression of CD14, CD34 and CD45 (Figure 1B).

Expression of pluripotency markers

The MAPC expressed pluripotency markers Oct-4 and Nanog at gene and proteins levels while MSC expressed none of these markers either at gene or protein levels (Figure 2).

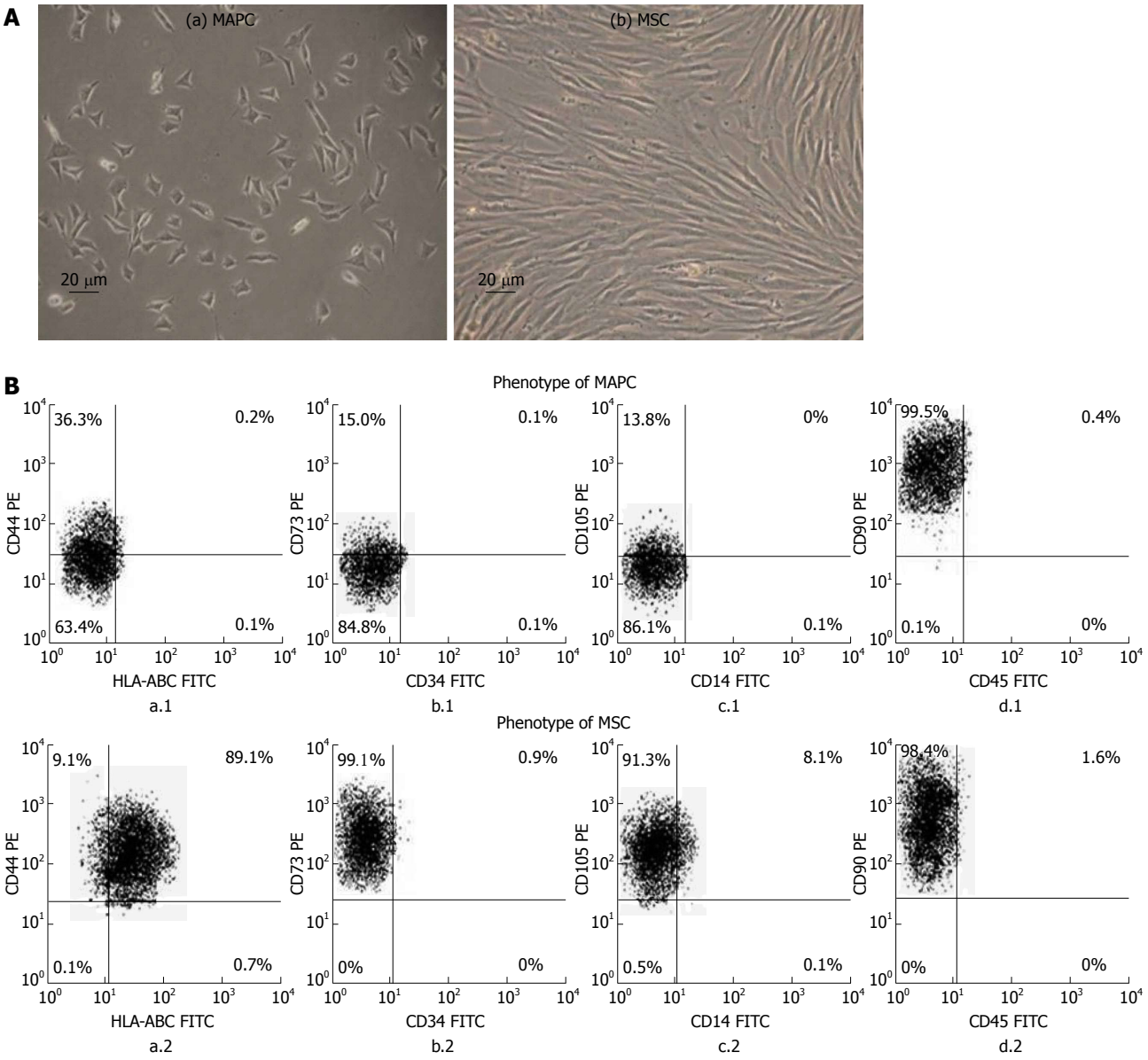

Figure 1 Morphology and phenotypic markers of multipotent adult progenitor cells and mesenchymal stem cells. A: Representative photomicrographs (×10, 20 μm) of primary cultures showing (a) small trigonal morphology of multipotent adult progenitor cells (MAPC) and (b) large spindle shaped morphology of mesenchymal stem cells (MSC); B: Representative flow-cytometric dot-plots showing expression of (a.1 and a.2) HLA-ABC (FITC)/CD44 (PE), (b.1 and b.2) CD34 (FITC)/CD73 (PE), (c.1 and c.2) CD14 (FITC)/CD105 (PE), and (d.1 and d.2) CD45 (FITC)/CD90 (PE) on MAPC and MSC, respectively. FITC: Fluorescein isothiocyanate; PE: Phycoerythrin.

Neuro-ectodermal differentiation efficiency

Following treatment with neurogenic induction medium, the cells which differentiated from both MAPC and MSC, had morphological characteristics of neuronal like cells as revealed by their bipolar elliptical shape and/or multiple branching points and neuritis (Figure 3A) and they expressed NF-200, MAP-2 and GFAP as revealed by immunocytochemistry (Figure 3B). The MAPC derived neuronal cells compared to those derived from MSC, showed a significantly higher fold change expression of NF-200 (0.64), GFAP (0.52), Tau (0.59), MAP-2 (0.72), Olig-1 (0.18) and NSE (0.29) ($P < 0.01$ for Olig-1 and $P < 0.001$ for rest) (Figure 3C). Similarly the fold change expression of *NF-200* (1.34), *GFAP* (1.12), *Tau* (1.08), *MAP-1B* (0.92), *MAP-2* (1.14) and *NSE* (0.4) genes were significantly higher in MAPC derived neuronal cells compared to those derived from MSC ($P < 0.001$ for all) but there was no difference in the fold change expression of *Olig-1* gene (0.08) in neuronal cells derived from both stem cell types ($P > 0.5$) as revealed by real time quantitative PCR (Figure 3D).

DISCUSSION

Our study shows that MAPC and MSC differ from each other in terms of morphology, phenotypic and pluripotency markers, and their neuro-ectodermal differentiation potential. Morphologically, MAPC are small trigonal cells while MSC are elongated spindle shaped cells. Phenotypically MAPC have no expression of HLA-ABC and

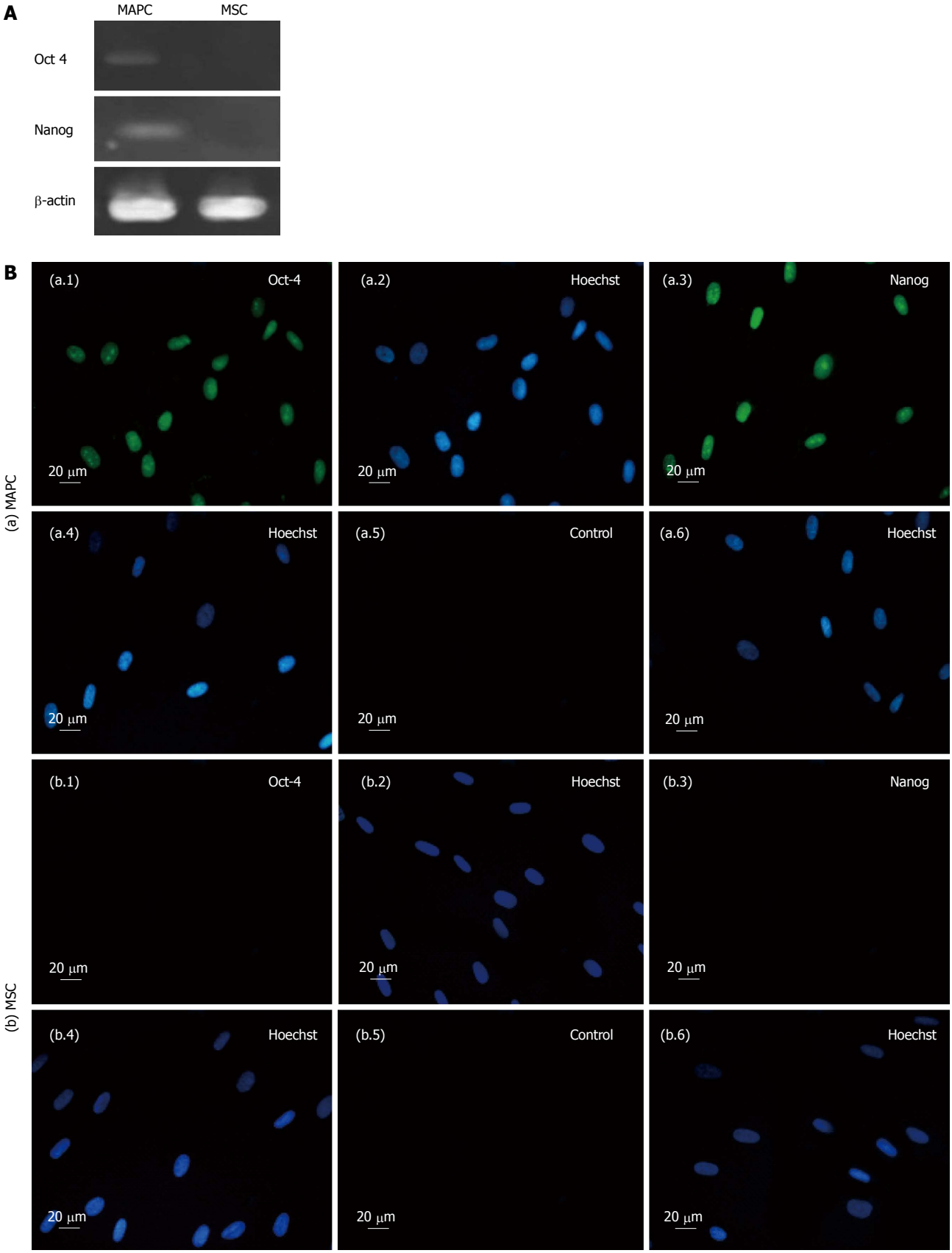

Figure 2 Expression of embryonic markers by multipotent adult progenitor cells and by mesenchymal stem cells. A: Expression of *Oct-4* and *Nanog* genes in multipotent adult progenitor cells (MAPC) and no expression of these genes in mesenchymal stem cells (MSC) as revealed by reverse-transcription polymerase chain reaction. β-actin represents the house keeping gene; B: Representative Immunocytochemistry photomicrographs (×40, 20 μm) of (a) MAPC showing fluorescein isothiocyanate (FITC) and Hoechst staining for Oct-4 (a.1 and a.2, respectively), Nanog (a.3 and a.4, respectively) and of controls, *i.e.*, cells with no primary antibody (a.5 and a.6, respectively); and (b) MSC showing FITC and Hoechst staining for Oct-4 (b.1 and b.2, respectively) and Nanog (b.3 and b.4, respectively) and of controls, *i.e.*, cells with no primary antibody (b.5 and b.6, respectively).

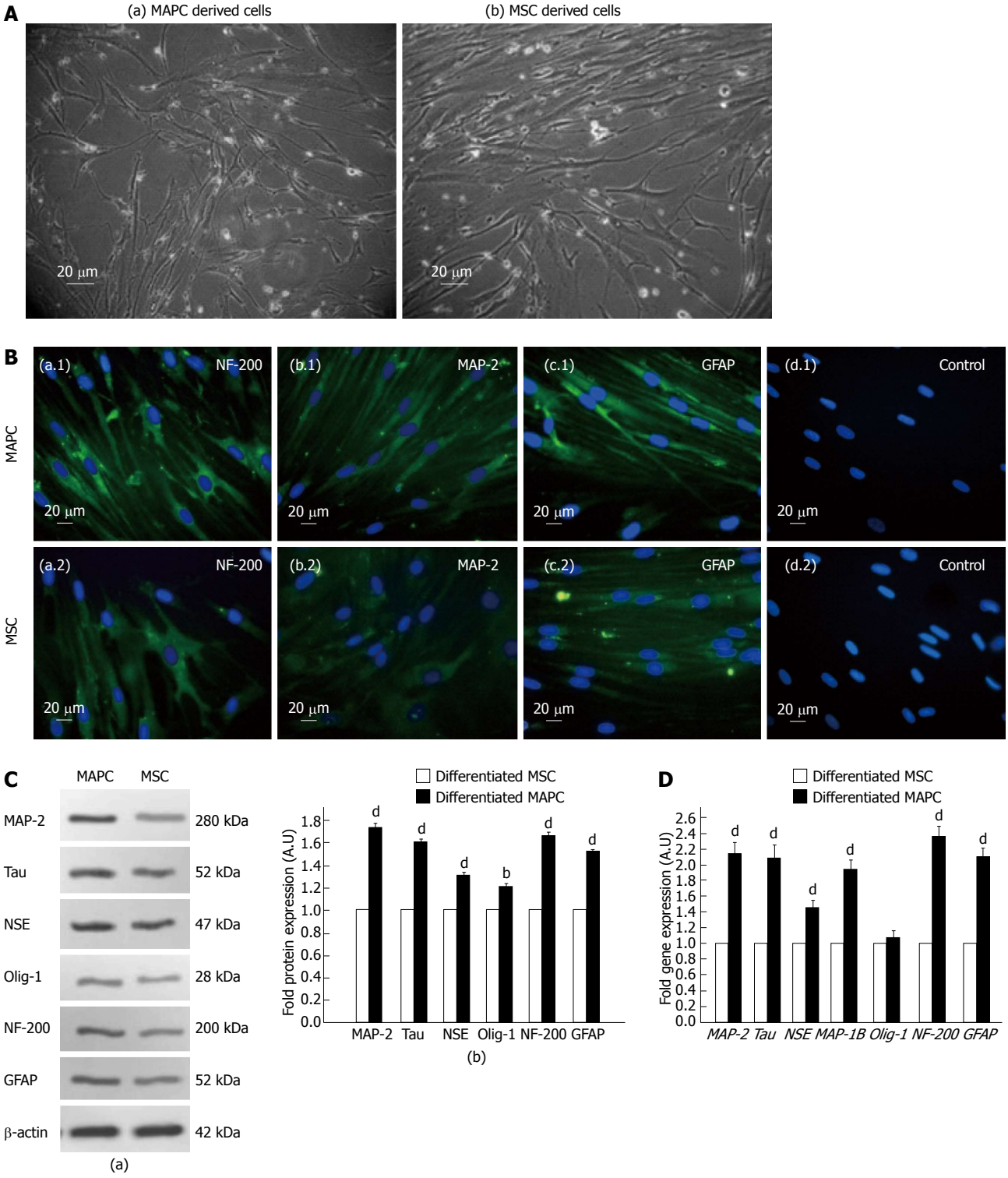

Figure 3 Neural differentiation potential of multipotent adult progenitor cells and of mesenchymal stem cell. A: Representative photomicrographs (×10, 20 μm) of neuronal cells differentiated from (a) multipotent adult progenitor cells (MAPC), and (b) mesenchymal stem cell (MSC); B: Representative immunocytochemical photomicrographs (×40, 20 μm) showing an overlay of fluorescein isothiocyanate (FITC) and Hoechst staining for (a.1 and a.2) neuronal filament-200 (NF-200), (b.1 and b.2) microtubule-associated protein 2 (MAP-2), and (c.1 and c.2) fibrillar acidic protein (GFAP) expression in neuronal cells differentiated from MAPC and MSC, respectively. Negative control represents cells with no treatment with neurogenic medium (d.1) MAPC and (d.2) MSC; C: Immunoblots analysis of neural proteins in MAPC and MSC derived neuronal cells. (a) Representative immunoblots of MAP-2, Tau, neuron-specific enolase (NSE), oligodendrocyte-1 (Olig-1), NF-200 and GFAP proteins in MAPC and MSC derived neuronal cells; (b) Error-bar diagrams showing fold change expression of proteins in MAPC (solid bars) and MSC (open bars) derived neuronal cells as revealed by densitometric quantification of immunoblots. $^{b}P < 0.01$, $^{d}P < 0.001$ *vs* MSC group; D: Error-bar diagrams showing expression of *MAP-2*, *Tau*, *NSE*, *MAP-1B*, *Olig-1*, *NF-200* and *GFAP* genes in MAPC (solid bars) and MSC (open bars) derived neuronal cells as revealed by real-time quantitative polymerase chain reaction. The values of MSC and MAPC treated with neurogenic medium and their untreated counterparts were normalized to housekeeping glyceraldehyde-3-phosphate dehydrogenase and the fold increase values of MAPC compared to MSC have been expressed as mean ± SE, $^{b}P < 0.001$ *vs* MSC group.

low expression of CD44, CD73 and CD105 while MSC possess high expression of all these markers. In addition, MAPC express Oct-4 and Nanog both at gene and protein levels but MSC lack expression of these markers. The MAPC have higher neuro-ectodermal differentiation potential than MSC as revealed by their significantly higher expression of NF-200, GFAP, Tau, MAP-2, Olig-1 and NSE proteins and *NF-200, GFAP, MAP-2, MAP-1B, Tau* and *NSE* genes. To the best of our knowledge this is the first study showing a parallel comparison of phenotype, pluripotency markers and neuro-ectodermal differentiation potential of MAPC and MSC isolated from the same samples of human bone marrow.

The existence of MAPC in the stroma of adult bone marrow has been described previously[1,3-5] by different groups and most of these studies support our observation of small triangular morphology of MAPC. However despite this morphological difference, there are no well defined phenotypic markers distinguishing MAPC from MSC. We have observed that MAPC have low expression of CD44, CD73, and CD105 and no expression of HLA-ABC, while MSC have high expression of these markers.

Human embryonic stem cells also have no or negligibly low expressions of HLA-ABC highlighting that MAPC have properties similar to embryonic stem cells[18]. We found that MAPC express pluripotency markers Oct-4 and Nanog both at gene and protein levels but MSC entirely lacked expression of these pluripotency markers. The expression of Oct-4 and Nanog on MAPC corroborates with expression of these and other pluripotency markers in ELSC[10], MASC[6] and MPC[7]. A few studies have reported that Oct-4, Nanog and other pluripotency markers are also expressed in MSC derived from bone marrow and other adult tissues[19] and one study has shown that MSC express Nanog but not OCT-4[20]. In another study, it has been shown that culture conditions of low serum content, induce expression of Oct-4, Nanog and other pluripotency markers on MSC[21]. We have cultured MSC under standard serum conditions, and thus the difference in expression of pluripotency markers between our and these studies may be due to difference in culture conditions which either have induced expression of Oct-4 and Nanog on MSC or promoted the growth of a population of MAPC in the cultures. Similar to our observation in MAPC, the expression of Oct-4, Nanog and other pluripotency markers has been shown in fetal MSC, but not in adult MSC[22]. Thus lower expression of the conventional mesenchymal markers, no expression of HLA-ABC and expression of Oct-4 and Nanog, may be used as suitable markers to distinguish MAPC from MSC.

Bone marrow derived MSC have been reported to exhibit trans-differentiation into cells of neuronal lineage, thereby claiming for a role of these stem cells in therapy for neurological disorders[15,16]. More recently, MAPC have been shown to differentiate into neuronal cells and promote neuronal regeneration[12,13]. However, no data exists on comparative analysis of neural differentiation potential of MAPC and MSC. In the present study, we have carried out a parallel comparison of neuro-ectodermal potential of MAPC and MSC at protein and gene levels. We studied both stem cell types for gene and protein expression of markers of axons (NF-200 and Tau), astrocytes (GFAP), dendrocytes (MAP-1B, MAP-2 and Olig-1) and neurons (NSE) and observed that MAPC show significantly higher expression of *NF-200*, *Tau*, *GFAP*, *MAP-1B*, *MAP-2* and *NSE* genes in comparison to MSC. Moreover, we compared the protein expression of NF-200, Tau, GFAP, Olig-1, MAP-2 and NSE, and similar to gene expression, we found significantly increased expression of these proteins in MAPC compared to MSC. Thus MAPC appear to have a greater predilection for neural differentiation, which needs to be therapeutically evaluated *in vivo* in pre-clinical animal models of neurological disorders.

In conclusion, our study showed that MAPC can be differentially characterized from MSC as Oct-4 and Nanog positive stem cells with no expression of HLA-ABC and low expression of mesenchymal markers CD44, CD73 and CD105 and they possess higher neuro-ectodermal differentiation potential than MSC indicating that MAPC may be more suitable cell type than MSC for cell based therapy for neurodegenerative disorders. Future studies directed towards the *in vivo* evaluation of the therapeutic potential of MAPC in pre-clinical models would lead to development MAPC based therapies for neurological diseases.

COMMENTS

Background

Multipotent adult progenitor cells (MAPC) and mesenchymal stem cells (MSC), the two predominant stem cell types of the bone marrow stroma, are currently being explored for cellular therapy of neurodegenerative disorders. However, there is no data on their phenotypic difference and it is also not yet known that which of these two possess a greater potential for neural differentiation and thus would be more suitable for therapeutic use.

Research frontiers

On the basis of a parallel comparison of the two stem cell types derived from the same sample of the bone marrow, this study reports that MAPC can be differentially characterized from MSC as Oct-4 and Nanog positive stem cells with no expression of human leukocyte antigen-ABC and low expression of other mesenchymal markers and with a greater predilection for differentiation into neuro-ectodermal lineage. Future studies comparing their *in vivo* therapeutic efficacy in pre-clinical animal models of neurodegenerative disorders would be important to confirm superiority of MAPC over MSC for therapeutic application in these diseases.

Innovations and breakthroughs

This is the first study reporting a parallel comparison of MAPC and MSC and demonstrating phenotypic differences between two stem cells types and a greater potential of MAPC than MSC towards neuro-ectodermal lineage.

Applications

This study offers a foundation for comparative studies on MAPC and MSC in experimental models of neurodegenerative disorders that in turn may lead to initiation of clinical studies on MAPC in these disease states.

Terminology

MAPC represent a primitive population of non-hematopoietic stem cells present in the bone marrow and other tissues. Their phenotype is not yet well defined but they express embryonal markers and give rise to cells of all the three germ layers.

Peer review

This is a great manuscript that compares MAPC with MSC. The presented data are significant to the field of research and adds to the knowledge about these stem cell types and their potential.

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05;

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