Hair follicle dermal cells repopulate the mouse haematopoietic system

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Summary

Skin and hair follicle stem cell biology is the focus of increasing interest, not least because the adult hair follicle has well defined dermal and epithelial populations that display distinct developmental properties. Recent evidence suggests that a number of adult cell populations have much broader stem cell capabilities than previously thought. To examine whether this applied to the hair follicle, and with a view to developing the follicle as a stem cell model system we investigated whether adult hair follicles were capable of demonstrating haematopoietic stem cell activity. To investigate haematopoietic activity in hair follicles we first used in vitro haematopoietic colony assays. This demonstrated that rodent hair follicle end bulbs as well as micro-dissected dermal papilla and dermal sheath cells actively produced cells of erythroid and myeloid lineages but that follicle epithelial cells did not. As a more stringent test, we then transplanted cultured dermal papilla or dermal sheath cells from transgenically marked donor mice into lethally irradiated recipient mice and observed multi-lineage haematopoietic reconstitution when assayed at intervals of up to one year. Colony assays from bone marrow of primary recipients revealed that over 70% of clonogenic precursors were derived from donor hair follicle cells. When bone marrow from primary mice was harvested and used to repopulate secondary myeloablated recipients, multi-lineage haematopoietic engraftment was observed. Our data show that dermal but not epidermal compartments of the adult hair follicle have much broader stem cell activities than previously described. Although the treatment for many forms of blood disorder, such as leukemia, often requires transplantation of haematopoietic stem cells (HSC), their availability can be rate limiting. Given its easy accessibility, our identification of the hair follicle as a source of extramedullary haematopoietic stem cell activity makes it an attractive potential source for blood stem cell therapeutics and highlights its value as a model system in adult stem cell biology.

Key words: Hair follicle, Dermal papilla, Dermal sheath, Haematopoietic stem cell

Introduction

All tissues in the mammalian adult are ultimately derived from stem cells. Until recently, these cell types have been grouped into two broad families: totipotent and lineage-restricted (somatic) stem cell types. Totipotent stem cells (such as embryonic stem cells) are characterized by their ability to contribute to every lineage in the body, as exemplified in vivo by the formation of germ-line competent chimaeras following injection into host blastocysts, or in vitro by differentiation into multiple lineages under selected tissue culture conditions. Adult somatic stem cells have also been characterized for many tissues (Spangrude et al., 1991; Gage et al., 1995; Watt, 1998) and although possessing substantial capacity to proliferate, it was believed that such cells were capable of differentiation into a limited number of cell types. In the past few years there has been increasing evidence that adult stem cells may have a far greater differentiation plasticity than previously thought. Bone marrow is capable of turning into skeletal muscle (Ferrari et al., 1998); skeletal muscle can turn back into blood (Gussoni et al., 1999; Jackson et al., 1999); brain can turn into blood (Bjornson et al., 1999) and back again (Eglitis and Mezey, 1997; Brazelton et al., 2000; Mezey et al., 2000); and bone marrow can turn into liver (Petersen et al., 1999; Theise et al., 2000a; Theise et al., 2000b; Henegariu and Krause, 2000; Lagasse et al., 2000). Nevertheless major questions remain in relation to the limits of plasticity in somatic cells and to what extent the observed reprogramming phenomena derive from cell sub-populations, from transformation events that have occurred within donor cell populations when in culture (Morshead et al., 2002), or as a result of cell fusion (Ying et al., 2002). One route to addressing these issues may be to examine those adult stem cell populations that are most specialised and/or have widest potential for differentiation. In this regard, bone marrow cells have been the model of choice for many researchers. Labelled bone marrow cells injected into adult mice have been shown to express proteins characteristic of neuronal phenotypes in the brain (Brazelton et al., 2000), and it was recently shown that a single bone marrow cell could populate multiple tissues of irradiated host animals (Krause et al., 2001). Also while the functional significance of many of the reprogramming/transdifferentiation experiments has still to be established, bone marrow cells have been shown to home to damaged target organs and replace injured tissues. For example, bone marrow cells transplanted into infarcted mycoardium were able to create new myocardial tissue (Orlic et al.., 2001), and they have been shown to ‘home’ to damaged muscle in irradiated dystrophic mdx mice (Ferrari et al., 1998; Gussoni, 1999). Bone marrow contains a mesenchymal stem

cell population that can divide and has the potential to differentiate in culture to many different mesenchymal lineages including bone, cartilage, adipocyte and muscle. By isolating small numbers of human bone marrow cells using density gradient centrifugation and showing that they could be induced to differentiate into adipocytic, chondrocytic or osteocytic lineages, Pittenger et al. provided good evidence that bone marrow contains a multipotent mesenchymal stem cell population rather than a mixture of committed progenitor populations (Pittenger et al., 1999).

However, if the plasticity of stem cells is ever to be used in therapeutics, ease of access to stem cell repositories will be very important and the provision of mesenchymal stem cells from bone marrow requires bone marrow extraction and cell sorting. Several research groups have investigated alternative sources of adult stem cells. Adipocytes obtained from subcutaneous fat by liposuction have been shown to produce osteogenic, chondrogenic and myogenic derivatives (Zuk et al., 2001), but no blood or nerve cells. Stem cells obtained from skin dermis can produce neural as well as mesodermal derivatives (Toma et al., 2001), but the origin of this stem cell population is unclear, as the initial dermal population is heterogeneous. However, cells of the hair follicle are highly accessible and can also be isolated as discrete populations.

Unlike some of the cell types whose stem cell potential is being investigated, hair follicle dermal cells derive from an organ that is unique in the adult mammal in terms of its range of developmental activities. There is increasing evidence that follicles contain stem cells that play key roles for skin as a whole, and within the follicle, the lineage relationships between the dermal cell populations is well defined (Taylor et al., 2000; Oshima et al., 2001). Hair follicle dermal cells are perhaps best considered as embryonic type cells retained in an adult system, and flexible in their range of activities, rather than specialised adult stem cells. The embryonic-like properties of the hair follicle dermal cells derive from the fact that they segregate from intrafollicular dermis relatively early on in follicle development and assume characteristic morphological and molecular phenotypes. Experimentally we have shown that adult hair follicle dermal cells have unparalleled capacity to induce hair growth when combined with different epidermal partners (Jahoda and Reynolds, 1996). Recently we transplanted follicle dermal sheath tissue from one person to another and showed that it induced follicles that grew hair without undergoing the rejection process normally associated with allografts (Reynolds et al., 1999). Therefore dermal sheath cells would appear to possess a degree of immunoprivilege that underlines their potential for use as universal donors in stem cell-based therapies. When the hair follicle base is experimentally amputated, residual cells of the dermal sheath (DS; Fig. 1) replace the main inductive dermal element the dermal papilla (DP), and restore hair growth in a unique example of mammalian regeneration. Thus there is also clear mesenchymal cell lineage transition from DS to DP cells, in this case (apparently) mediated by the follicle epithelium (Reynolds and Jahoda, 1996).

Follicle epithelial cells are increasingly studied in relation to skin stem cell and tumor biology. It has long been recognized that in wounded skin new epidermis can derive from skin appendages. However, recent work using sophisticated cell labeling techniques provides evidence that follicle epithelial stem cells are involved in the continuous physiological process of epidermal replacement in normal healthy skin (Taylor et al., 2000). Interactions between adjacent cells of different types are a major theme in many areas of developmental biology. In mammals, interactions between cells derived from the embryonic ectoderm and mesoderm are central to the development of many structures, including skin, teeth, mammary glands and the digestive and nervous systems. The mammalian hair follicle provides a particularly useful model for studying these phenomena (Kratochwil et al., 1996). Follicles are conveniently located in the skin of the animal. The developmental interactions persist into adulthood, in the course of wound repair and normal follicle growth cycling. Most importantly, hair follicles contain readily identified populations of interacting cells that are clustered in discrete sites. Each cell type can be isolated and continues to show pronounced interactive abilities when cultured in vitro. Thus the hair follicle is turning out to be not only a key stem cell repository within skin, but also a major developmental model. Further, given that access to stem cells of the skin is a relatively simple procedure (biopsy) compared with stem cells of (for example) the brain or bone marrow, the therapeutic potential and plasticity of follicle stem cells needs to be explored. In our laboratory it was previously observed that hair follicle dermal papilla and dermal sheath cultures generated cells expressing a variety of blood cell markers (A. J. Reynolds, personal communication). Moreover, the collagen capsule of vibrissa follicles encloses sinus cavities, and after extended organ culture of totally isolated follicles we observed the accumulation of high numbers of non-erythrocyte blood cells adjacent to the dermal sheath within these structures. These observations indicated that endogenous haematopoietic progenitor activity could occur within the dermal compartment of the follicle. Given that the therapeutic need for alternate sources of haematopoietic stem cells is most pressing, we set out to investigate directly the haematopoietic potential of these hair follicle dermal populations. In this paper we report that the follicle dermal sheath and dermal papilla show haematopoietic activity in vitro and cells cultured from these structures can contribute to the regeneration of the entire haematopoietic system of lethally irradiated mice in vivo.

Materials and Methods

Isolation of follicle end bulbs and individual components

Follicle end bulbs were isolated as previously described (Reynolds and Jahoda, 1991). In brief, the proximal tips of adult Zin40 mouse vibrissa follicles, exposed on the internal surface of mystacial skin flaps, were removed and placed in Eagle's minimal essential medium (MEM; Gibco BRL) containing antibiotics. To isolate dermal and epithelial components from the end bulb, the outer collagen capsule was inverted and the dermal sheath (DS) layer, and epidermal matrix were then delicately eased away from around the dermal papillae (DP). Papillae were isolated by basal stalk severance and the remaining cups of DS tissue detached from the collagen capsule.

Establishment and maintenance of DP and DS cell cultures

Cell culture was performed as previously described (Jahoda and Oliver, 1981). Around a dozen papillae, or pieces of sheath tissue were pooled and cultured in 35 mm Petri dishes. All explants were initially maintained in MEM supplemented with 20% fetal calf serum (FCS, Gibco BRL; Renfrew, UK) at 37°C, 5% CO_2. The cultures were

routinely passaged as described elsewhere (Reynolds and Jahoda, 1991).

Colony forming unit (CFU) assays

CFU-GEMM assay

CFU-GEMM assays were performed according to the manufacturer's instructions (Stem Cell Technologies; Vancouver, Canada), In brief, 3×10^4 cells in a total volume of 0.3 ml were added to 3 ml of Methocult medium yielding triplicate cultures of 1.1 ml each. Methocult containing cells was dispensed into three 35 mm dishes using a 16G blunt-end needle. Two plates were then placed into a 100 mm covered dish containing a third uncovered dish containing 3 ml of sterile water. Cultures were then placed in a 37°C incubator maintained with 5% CO_2 and >95% humidity. The concentration of cytokines in Methocult is as follows: IL-3 (10 ng/ml); IL-6 (10 ng/ml), SCF (50 ng/ml), Epo (3 units/ml). Dissected parts from the hair follicle were placed on top of methocult (7-8 in total) and plates were incubated as above.

CFU-A assay

The in vitro CFU-A assay was set up as described previously (Pragnell et al., 1988). In brief, a feeder layer consisting of 0.6% agar in a modified Eagle's medium (α-MEM; Gibco BRL) with conditioned medium from two cell lines (AF1-19T, a source of granulocyte macrophage colony stimulating factor, GM-CSF; and L929, a source of M-CSF) was poured into 3 cm diameter tissue culture grade dishes (1 ml per layer). Dissected DP and DS (5-6 per plate) were placed on top of the 0.3% agar and α-MEM mixture to form the upper layer. When DP or DS cells were assayed, 3×10^4 cells were added to 0.3% agar in α-MEM and plated to form an upper layer. The dishes were incubated for 11 days at 37°C in a humidified atmosphere with 5% O_2 and 10% CO_2. The presence or absence of haematopoietic progeny was determined by the formation of mixed colonies of neutrophils and macrophages with diameters greater than 2 mm after 11 days of incubation. The mixed nature of the developing colonies and the high proliferative capacity of the clonogenic cell, alongside data on radiation sensitivity, anatomical location and sensitivity to mitotic poison indicates that this assay detects a primitive haematopoietic cell analogous to CFU-S (Pragnell et al., 1988; Graham et al., 1990).

Stem cell transplantation

DP and DS cells from Zin40 mice cultured as described above were harvested by trypsinisation. 20,000 cells were mixed with 200,000 nucleated bone marrow cells from Balb/c mice and injected intravenously into recipient Balb/c mice that had received 1.2 Gy of γ-irradiation. Recipients received prophylactic aureomycin in drinking water. For transplantation into secondary recipients, bone marrow was harvested from the primary mice and 2×10^6 nucleated cells were injected into each secondary Balb/c recipient, prepared as described above.

Analysis of transplant recipients

Bone marrow, spleen and peripheral blood were collected from both primary and secondary sacrificed recipients and DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Southampton, UK). Thirty five cycles of 94°C (1 minute), 62°C (1 minute) and 72°C (1 minute) were used to amplify lacZ from 100 ng of genomic DNA with the following primer pair: 5′-CGCTCACATTTAATGTTGATGAAAGC and 5′-TCCAGATAA-CTGCCGTCACTCCAA. A second PCR was performed with murine Wnt8b specific primers 5′-AACGTGGGCTTCGGAGAGGC and 5′-GCCCGCGCCCTGCAGCAGGT as an internal control.

Results

The proximal tips of adult mouse and rat vibrissae follicles were assessed for haematopoietic potential by two in vitro colony forming unit assays, CFU-GEMM and CFU-A. We initially dissected the proximal tips of the vibrissae follicles into three parts: end bulbs, medium and top (Fig. 1) and subjected them to CFU-GEMM assay (Fig. 2). In three independent experiments 100% of the mouse and rat end bulbs produced macroscopic colonies containing cells of erythroid and myeloid lineages (Fig. 2A). 68% of the medium parts of vibrissae also produced macroscopic colonies containing cells of myeloid lineages, but not erythroid cells (Fig. 2B), while only 11.2% the top part of the vibrissae showed some small colonies containing cells of the myeloid lineage (Fig. 2C). To identify which follicle structures contained haematopoietic potential we then micro-dissected the end bulb of mouse and rat vibrissae into their constituent parts: epithelium, dermal sheath (DS) and dermal papilla (DP), and assessed each individually by CFU-GEMM assay. Mixed haematopoietic colony formation was found to be associated exclusively with the DS (Fig. 2D) and DP (Fig. 2E). No haematopoietic activity was observed with the epithelial structures (Fig. 2F). We then repeated the same experiments using the second assay, CFU-A, which identifies a primitive haematopoietic precursor, equivalent to CFU-S (Pragnell et al., 1988). Similarly to the CFU-mix, 100% of end bulbs (Fig. 3A) and 70% of the medium part of vibrissae produced haematopoietic colonies containing granulocytes and neutrophils in the CFU-A assay; however, only 14.2% of the top parts showed colony formation (Table 1). Dissection of DP and DS from both mouse and rat end bulbs indicated that haematopoietic activity was closely associated with them (Fig. 3B,C,E,F) and not with the epithelial component. In contrast, aorta and peripheral blood failed to generate haematopoietic colonies when tested using the same assay.

We then established primary explant cultures of DS and DP

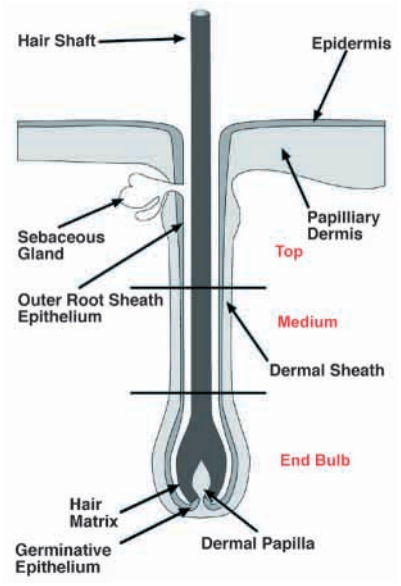

Fig. 1. Schematic presentation of the hair follicle. Black lines indicate the plane of dissection used to separate the end bulb, medium and top section of the vibrissa (see Fig. 2).

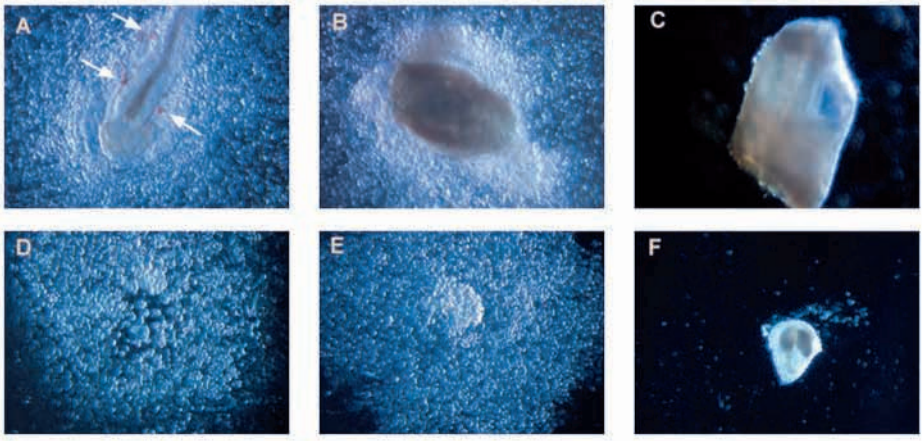

Fig. 2. Follicle tissues display haematopoietic activity in vitro. CFU-GEMM assay results show colony formation from the end bulb of the mouse vibrissa (A), the medium section of the mouse vibrissa (B), the top section of the mouse vibrissa (C), the dermal sheath dissected from the mouse end bulb (D), the dermal papilla dissected from the mouse end bulb (E) and the epithelium dissected from the mouse end bulb (F). Haemoglobinised cells produced in the assay are indicated by arrows.

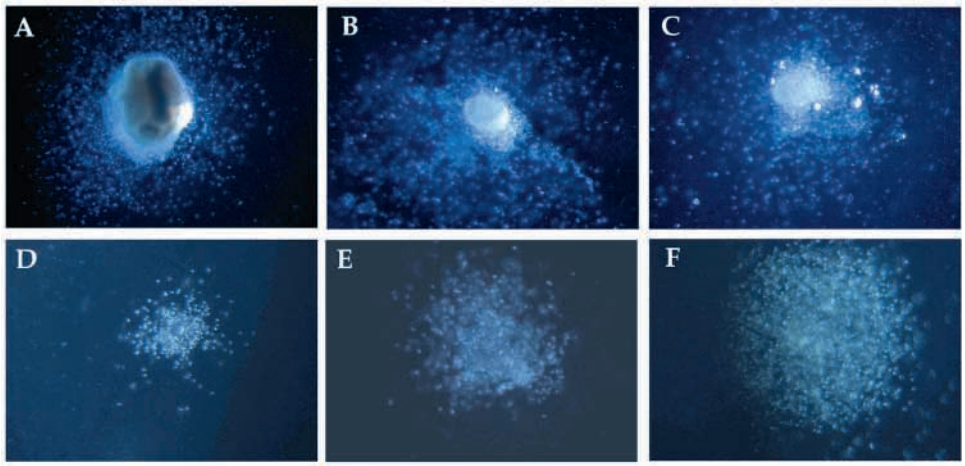

Fig. 3. Mouse and rat follicle tissues display haematopoietic activity in vitro. CFU-A assay results from proximal tip of rat vibrissa follicle (A); dermal sheath of rat vibrissa follicle (B); dermal papilla of rat vibrissa follicle (C); proximal tip of mouse vibrissa follicle (D); dermal sheath of mouse vibrissa follicle (E); and dermal papilla of mouse vibrissa follicle (F).

cells and carried out CFU-A assays on cells passaged from 1 to 3 times (Fig. 4A,B). Both DS and DP cell cultures displayed haematopoietic activity as shown by the production of large haematopoietic colonies in CFU-A assays that were indistinguishable from those formed by conventional bone-marrow-derived progenitors (Fig. 4C,D). Flow cytometry of the DP or DS cultures with anti-CD45 monoclonal antibody (PharMingen, San Diego, CA) was unable to detect pre-formed haematopoietic cells at this level of analysis; however, CD45 cells were detected in the colonies formed in the CFU-A assays. Coupled with the comparatively small number of haematopoietic colonies formed (approximately 1 in 6000 DP or DS cells plated in CFU assay), these data suggest that if pre-formed haematopoietic progenitors are present there must be only relatively small numbers in each follicle.

Although the CFU data we obtained from both the dissected DP and DS and their respective cultures strongly suggested the presence of primitive haematopoietic potential, these in vitro assays detect haematopoietic progenitors that include, but are not exclusively, HSC. To determine the presence of HSC activity in these cultures, we assessed their capacity to generate long-term multi-lineage haematopoietic reconstitution of myeloablated recipient animals. For this we used a competitive repopulation transplantation assay (Harrison, 1980), in which a given cell population is required to compete in the same recipient with a genetically distinguishable standard source of haematopoietic stem cell activity. Nucleated cells from unfractionated bone marrow of Balb/c mice (2×10^5 cells per animal) and cultured DS or DP cells from Zin40 mice (2×10^4 cells per animal) were injected intravenously into lethally irradiated Balb/c recipients. Zin40 mice were used as the source of donor tissue because they carry a transgene (LacZ); thus cells derived from the hair follicle could be distinguished from host or carrier bone marrow by genotype. Analysis using PCR is preferable to flow cytometry studies using fluorescein di-galactoside since our experience has shown high background staining of mouse tissues using this technique. Between 4 and 5 months post-irradiation we sacrificed eight transplanted mice and extracted DNA from the spleen, bone marrow and peripheral blood. PCR analysis for lacZ indicated the presence of dermal-derived cells in the recipient peripheral blood, spleen and bone marrow (Fig. 5A; Table 2). A semi-quantitative analysis of lacZ detection using variable percentages of transgenic and wild-type splenocytes show that this assay can detect low percentages of lacZ-labelled cells against a wild-type background (Fig. 5C). We sacrificed one

Table 1. Comparison of CFU-MEGG and CFU-A results for sections of mouse vibrissa follicles

	CFU-mix	CFU-A
End bulb	100%	100%
Medium section	68%	70%
Top section	11.7%	14.2%

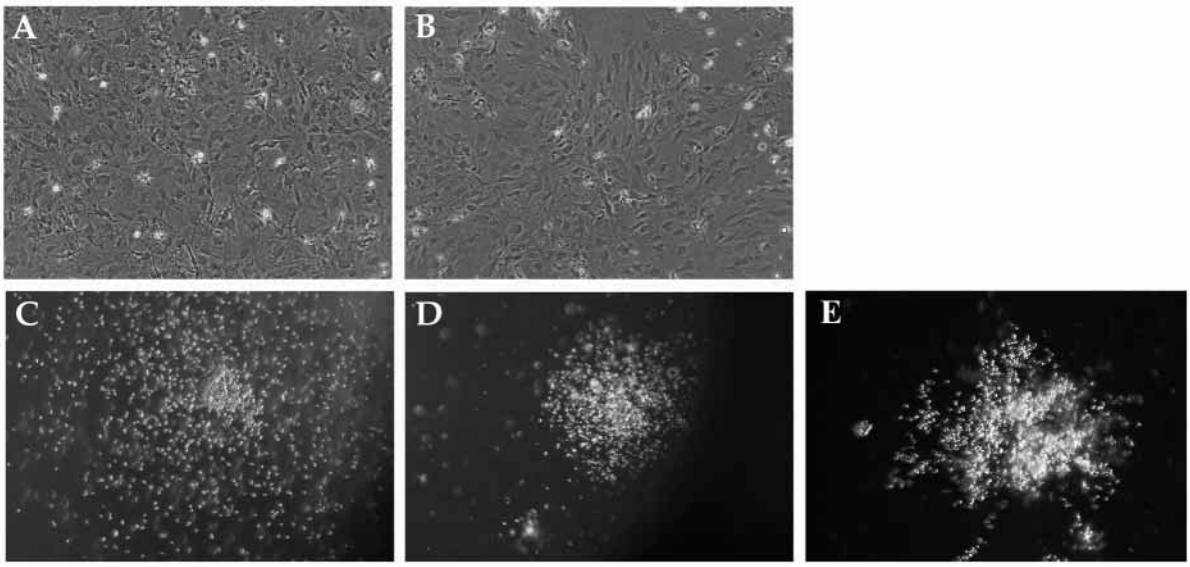

Fig. 4. DP and DS cultured cells display haematopoietic activity in vitro. DS and DP explants were cultured in vitro (A and B, respectively) and subjected to CFU-A assay (C and D, respectively). For comparison, E shows a CFU-A colony formed following plating of bone marrow cells.

recipient mouse 13 months after transplantation and found the presence of dermal-derived cells in the peripheral blood, spleen and bone marrow. We also sorted splenocytes from the recipient females into different subpopulations (e.g. B cells, T cells, myeloid cells) using immunomagnetic cell sorting (Miltenyi Biotech) and examined each for the presence of the transgene. Dermal-derived cells were found to have contributed to all haematopoietic lineages studied (Fig. 5B). The proportion of clonogenic haematopoietic precursors that were derived from the transgenic dermal cultures was then assessed by limiting dilution CFU-A analysis of recipient bone marrow. In three recipients that had received a mixture of DP and bone marrow cells, 72%, 73% and 75% of colonies formed from their bone marrow were found to be transgenic by PCR. Similarly, with two recipients that had received a mixture of DS and bone marrow cells, 80% and 77% of the colonies were transgenic. Although in our opinion this is strong evidence that cells of the DP and DS contain HSC activity, we then tested this further by transplanting bone marrow from the primary recipients into myeloablated secondary recipients as a robust test of stem cell competence. PCR analysis of the peripheral blood, spleen and bone marrow of these secondary mice 8 weeks post-irradiation indicated the presence of dermal-derived cells (Table 2). We have also found transgenic haematopoietic cells in all leukocyte populations that have been tested from these secondary recipients. Moreover, PCR analysis on single CFU-A colonies from the bone marrow of two secondary mice showed that 58% and 77% of colonies respectively were lacZ positive.

Discussion

The data presented show that cells derived from the dermal structures of the hair follicle have intriguing haematopoietic potential. Cells within the DS and DP generate haematopoietic colonies in vitro, and can contribute to all blood lineages in vivo for at least 13 months post-transplantation. These transplanted cells retain this regenerative potential when transferred to secondary recipients indicating their primitive stem cell nature. This follicle-derived activity appears to be general since we obtained similar in vitro results using rat and mouse follicle tissues. Therefore, in relation to therapeutic

Table 2. Summary of in vivo work

No. of recipients	Source of cells	No. of surviving recipients	PCR results
13 primary recipients*	Cultured DS cells from Zin40 donors	5	4 analysed up to 5 months after transplantation show incorporation of donor cells in the bone marrow, spleen and peripheral blood
6 primary recipients*	Cultured DP cells from Zin40 donors	6	5 analysed up to 1 year after transplantation show incorporation of donor cells in the bone marrow, spleen and peripheral blood
4 secondary recipients*	BM cells from primary recipients that had been transplanted with cultured DS cells from Zin40 donors	4	2 analysed 2 months after transplantation show incorporation of donor cells in the bone marrow, spleen and peripheral blood
4 secondary recipients*	BM cells from primary recipients that had been transplanted with cultured DP cells from Zin40 donors	4	2 analysed 2 months after transplantation show incorporation of donor cells in the bone marrow, spleen and peripheral blood

*Equal numbers of Balb/c mice were lethally irradiated but not injected: none survived.

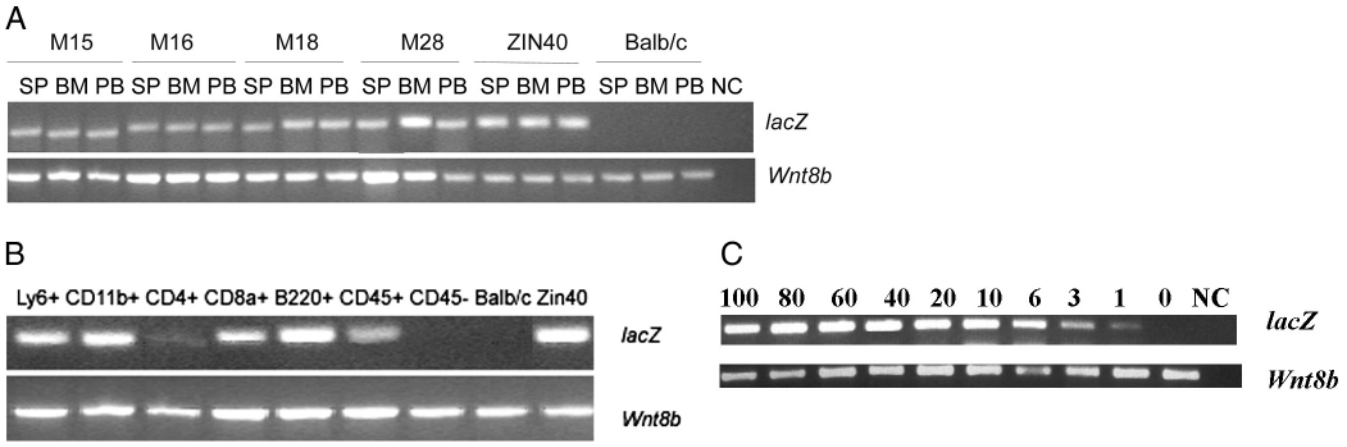

Fig. 5. (A) PCR analysis shows that cells from the follicle dermis reconstitute bone marrow, spleen and peripheral blood of recipients. The lacZ gene was detected in the peripheral blood, bone marrow and spleen of the Zin40 donor mouse, but not in the control recipients (Balb/c). M15 and M16 were transplanted with 2×10^4 Zin40 dermal sheath cultured cells and 2×10^5 Balb/c bone marrow cells. M18 and M28 were transplanted with 2×10^4 Zin40 dermal papilla cultured cells and 2×10^5 Balb/c bone marrow cells. NC, no DNA control. (B) Dermal-derived cells contribute to all haematopoietic lineages studied: confirmation by PCR. (A) Splenocytes from Balb/c recipient animals transplanted with dermal cells derived from LacZ transgenic animals (Zin40) were sorted into subpopulations (total leukocytes, CD45+; non-haematopoietic nucleated cells, CD45–; B cells, B220+; T cells, CD4+/CD8a+; and myeloid cells, CD11b and Ly6) and presence of transgenic leukocytes determined by PCR. A second PCR was performed with Wnt8b primers as a loading control. (C) Detection of transgenic donor cells by PCR. Mixtures of splenocytes from transgenic and non-transgenic animals were mixed in differing ratios from 100% transgenic to 0% transgenic and subjected to PCR for LacZ and Wnt8b.

provision, our finding of haematopoietic regeneration from a readily accessible source is highly significant. We can then begin to ask where the precise cellular origin of this phenomenon originates?

The hair follicle has been shown to be associated with differentiated blood cells in situ such as $CD4^+$ and $CD8^+$ T cells, Langerhans cells, macrophages and mast cells (Christoph et al., 2000). It is also vascularised so there is a finite possibility that what we observed was due to the presence of haematopoietic stem cells carried in the peripheral blood or, potentially, recruited from the endothelial cells themselves. To address this, we investigated the haematopoietic potential of aorta and peripheral blood and they both failed to show haematopoietic activity in our in vitro assays. In view of this data and the high proportion of follicles demonstrating haematopoietic activity it is likely that what we have seen with the hair-follicle-derived cells is a product of endogenous activity for which there are at least three possible explanations, none of which can be entirely excluded. One is that the apparently lineage-restricted dermal cells undergo a general reprogramming, possibly via extracellular cues from the new microenvironment into which they are introduced. There are many recent reports involving adult cell populations once considered to be lineage restricted, showing greater differentiation plasticity, several of which have involved haematopoietic stem cell activity (Jackson et al., 1999; Gussoni et al., 1999; Bjornson et al., 1999). Our current observations tend to argue against a general reprogramming event. Although nearly all follicles demonstrated endogenous haematopoietic activity, the fact that 1 in 6000 of cultured cells plated in the CFU experiments had haematopoietic potential suggests that these cells may be quite rare within each follicle. In spite of this, the dermal cultures appear to be at least as potent as conventional bone marrow, the high percentage of follicle-derived clonogenic cells in recipient animals suggesting, indeed, that these cell types may be capable of out-competing conventional bone marrow HSC. This result is similar to that found in work with stem cells obtained from skeletal muscle (Jackson et al., 1999). Another alternative is that follicles contain a small resident population of haematopoietic stem cells. Although we are confident that the follicle-associated HSC activity is not a consequence of residual peripheral blood within the dermal structures, we cannot exclude the possibility that the activity is a result of prior HSC recruitment from conventional haematopoietic sites. In this context, a recent report has shown that adult muscle-derived haematopoietic stem cell activity emanates from cells that are haematopoietic in origin (McKinney-Freeman et al., 2002). Our current work is aimed at distinguishing this possibility from the first one, and from the third option, which is that there are small numbers of permissive stem cells resident in the hair follicle dermis that possess multi-potency. In this context a single bone-marrow-derived stem cell has been shown to contribute to a number of different organ systems (Krause et al., 2001). Moreover, it was recently reported (Toma et al., 2001) that stem cells derived from skin dermis could be grown and differentiated in culture to produce multiple differentiation products including neurons and glia. This work suggests that there may be a multipotent stem cell present in the dermis of skin whose origin is unknown. Within skin, the hair follicle is seen as providing a discrete well-protected niche, and the follicle epithelium has recently been the focus of much skin stem cell and tumor biology research (Taylor et al., 2000; Oshima et al., 2001; Jahoda and Reynolds, 2000). In the current work we found no evidence of haematopoietic activity from epithelial tissue isolated from the lower follicle. Moreover, the sections of follicle above the base, which

incorporate the main epithelial stem cell compartments including the bulge region, revealed a reduced haematopoietic response compared with the end bulb. This highlights the follicle dermis as a separate location for progenitor activity. In a recent paper we suggested that, just as follicle epithelial cells are a major stem cell source for skin epidermis in wound healing, so follicle dermal cells might play the equivalent role in replacing dermal cells in dermal repair (Jahoda and Reynolds, 2001). In the light of recent evidence that follicle epithelial stem cells are involved in the continuous physiological process of replacement of epidermis in normal healthy skin (Taylor et al., 2000), we also hypothesised that follicle dermal cells might be involved in regulating the cellularity of normal dermis. The haematopoietic activity evident in the current study now gives rise to the idea that, within skin, the protected environment of the hair follicle is a key repository for a broad range of stem cell activities. In this context it is interesting that in the paper describing neuronal cell differentiation from skin dermis (Toma et al., 2001), all the skin used for the experiments contained hair follicles.

Our observation that hair follicles possess cells with endogenous haematopoietic activity could have considerable dermatological significance. There is growing interest in hair follicle immunology and evidence that the follicle has a unique immunological profile (Paus et al., 1999; Gilliam et al., 1998; Westgate et al., 1991). Therefore, it is possible to envisage that the capacity to produce haematopoietic cells locally in skin could play a role in, for example, wound healing and immune surveillance. This might be true, especially in view of the recent reports that the hair follicle can serve as an immediate reservoir of Langerhans cells between bone marrow and epidermis (Gilliam et al., 1998). By contrast, if it turns out that the follicle harbors a few multi-potent stem cells, then the effects that we have observed could be just a manifestation of the different environmental conditions that these cells are being put into. In any event, this does not detract from the essential fact that hair-follicle-derived cells can be turned into blood for practical purposes.

The implications for such new sources of HSC for potential therapy have been highlighted elsewhere (Saba et al., 2000). However, follicle-derived HSC activity may be of particular relevance to the human condition. It is now well established that rodent hair follicles display immune privilege, and our work and that of others has indicated that this is true for human dermal sheath cells (Paus et al., 1999; Reynolds et al., 1999); such cells may therefore prove to be admirable donor cell types in stem-cell-based therapies. Further, the ability to readily access dermal cell populations by skin biopsy, rather than a potentially more invasive procedure required for other stem cell types may make them particularly appropriate as a source of autologous or allogeneic HSC activity.

Note added in proof

Recent observations indicate that some haematopoietic activity may not be seen under in vitro culture conditions that promote hair growth in vitro.

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