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Blood-Borne Stem Cells Differentiate into Vascular and Cardiac Lineages During Normal Development

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ABSTRACT

Recent investigations have indicated that hematopoietic stem cells (HSCs) have the potential to differentiate into multiple non-blood cell lineages and contribute to the cellular regeneration of various tissues and multiple organs. Most studies to date on HSC potential have examined the adult, focusing on their potential to repair tissue under pathological conditions (e.g., ischemic injury, organ failure). Comparatively little is known about the physiological role of HSCs in normal tissue homeostasis in the adult, and even less of their contribution to organogenesis during prenatal development. This study reports the contribution of blood-borne cells to various organ systems of the developing embryo using a quail–chick parabiosis model. Under these conditions, the developing circulatory systems fuse between ED6–ED8, resulting in free exchange of circulating cells. Cells of quail origin, identified by quail-specific antibodies at ED15, were found in numerous organs of the parabiotic chick embryo. Circulating cells contributed to developing vasculature, where they differentiated into endothelial, smooth muscle, and adventitial tissues. In the heart, differentiation of circulating cells into cardiomyocytes was demonstrated using double immunolabeling for QCPN and sarcomeric actin or myosin. These results were confirmed by intramyocardial injection of quail bone marrow cells that were found to express markers of myocytes, coronary smooth muscle, and epicardium. Experiments using lacZ-transgenic chick embryos for a second positive cellular marker showed that fusion between chick and quail cells was a rare event. These results suggest that during development, multipotent cells are present in the embryonic circulation and home into different organs where they undergo tissue-specific differentiation. Moreover, the demonstration that blood-borne cells contribute to the development of various organs lends credence to claims that hematopoietic stem cells have utility for treating diseased or damaged tissues in the adult.

INTRODUCTION

STEM CELLS ARE SELF-RENEWING, nondifferentiated cells that serve as the starting cellular material for all the tissues in the body. Hematopoietic stem cells (HSCs) are multipotent cells in the bone marrow and circulation that give rise to differentiated blood cells. Also found in bone marrow are mesenchymal stem cells (MSCs) that are the precursors for stromal cell types, including fibroblasts and myocytes. Various studies have used transplantation

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of stem cells into the myocardium in attempts to restore cardiac function following myocardial infarction or other cardiac disease (1–7). It has been reported that transplanted donor HSCs and MSCs differentiate into cardiomyocyte-like cells in healthy myocardium (3,4,8). It has been suggested that these observations may not be a result of stem cell differentiation, but instead are due to cell fusion (9). Some recent studies (10,11) that have failed to replicate these early promising results have put into question the magnitude of stem cell potential.

Recently, a population of stem cells capable of differentiating into both cardiomyocytes and coronary vascular lineages was identified in human bone marrow (12), further supporting significance of this biological phenomenon. All of these studies were performed in adult models, with little information, apart from pioneering work of Zanjani and associates (13,14), on their potential in fetal development.

Despite the evidence that endogenous cardiac progenitors reside in the adult heart, regenerate cardiomyocytes functionally, and integrate into the existing heart circuitry (15), it is unclear whether these cells derive from the original heart field or are imported later in development from other extracardiac sources such as the circulation. Therefore, a quail–chick parabiosis model (16) was employed to study the contribution of blood-borne stem cells to various organ systems of the developing embryo. In addition, the potential of these cells to contribute to cardiac lineages by intramyocardial injections of quail bone marrow cells into fetal chick hearts was investigated. It was found that circulation-derived cells contributed to multiple organ systems, mostly in association with the forming vascular network. In the heart, evidence of their differentiation into cardiomyocytes and coronary smooth muscle cells was found and confirmed by results form bone marrow injection experiments. These findings establish circulation as a novel extracardiac cell source contributing to formation of the heart, which could be potentially used for designing new cell-based therapeutic strategies.

MATERIALS AND METHODS

Quail-chick parabiosis

Fertilized quail and White Leghorn chicken eggs were obtained from commercial vendors (Manchester Farms, Dalzell, SC, and Goldkist Hatchery, Sumter, SC, respectively). Quail-chick parabiosis was performed according to a modified version of the previously described protocols (16). Briefly, both embryos were allowed to develop separately for 2 days in a humidified incubator at 37.5°C. The shell and the membrane covering the air sac of the chick egg were carefully removed before inserting an 18-gauge needle connected to a 20-ml syringe to the albumen. Next, 12–14 ml of albumen was withdrawn once the tip of the needle was tightly positioned against the inner surface of the shell to avoid damaging the yolk. Subsequently, the narrow end of the parabiotic quail partner was cut open and 1–2 ml of albumen was removed without damaging the yolk using a 20-gauge needle connected to a 10-ml syringe. The developing quail embryo was combined with the chick embryo by pouring the remaining contents of the quail egg into the host chick shell. The opening of the chick shell hosting combined quail–chick embryos was tightly sealed with tape and the eggs were placed back into the incubator to allow development for additional 13 days.

Both quail and chick embryos were sampled at ED15. After removal from the host chick shell, the chick–quail parabiotic embryos were fixed in Dent's fixative, consisting of 20% dimethylsulfoxide (DMSO) and 80% methanol. For both embryos, the head, chest, and lower body were dissected separately and embedded in paraffin. Ten-micron sections were cut in series for immunohistochemical analyses. Experiments involving chick embryos heterozygous for nuclear-localized LacZ (17) were performed in the same manner. The quail member of the parabiotic pair was fixed by perfusion with 4% paraformaldehyde and then processed for whole-mount lacZ staining as previously described (18). After staining, the hearts were photographed and processed for paraffin sectioning.

Bone marrow cell injection

Bone marrow of ED14 quail embryos was isolated as described previously (19). The number of cells in the suspension was counted using a hemocytometer, and the final concentration of the cell suspension was adjusted to 5×10^6 cells/ml for injection. A volume of 10 μl was pressure-injected (Picospritzer, World Precision Instruments) into ventricular myocardium of ED8 chick embryos that were gently lifted from the eggs using a fine glass hook. Sampling was performed at ED18, with fixation and tissue processing as described above.

Immunohistochemistry

For orientation purposes, guide series at 200-micron intervals were stained with Hematoxylin & Eosin. Single immunostaining with the QCPN quail nuclear marker or QH1 quail vascular and hemangioblast marker (undiluted and 1:100, Developmental Studies Hybridoma Bank) detected by the peroxidase-diaminobenzidine method (Pierce) was performed to detect presence of

quail cells in the chick tissues, as well as to examine normal staining patterns within matched quail tissues. The co-expressions of selected tissue markers with QCPN was examined on tissue sections from both chick and quail parabiotic embryos. After deparaffinization, permeabilization, and blocking with 1× TBSA-BSAT (Tris buffer saline with 0.1% sodium azide and 1% bovine serum albumin and 0.1% Triton-X), double immunohistochemistry staining was performed using quail-specific QCPN antibody (undiluted; Developmental Studies Hybridoma Bank; detected with 1:100 Cy3-coupled goat anti-mouse IgG, Jackson Immuno) with one of the following primary antibodies: sarcomeric actin (1:1,000, Sigma) or myosin heavy chain (MF20, undiluted, Developmental Studies Hybridoma Bank) for myocytes, smooth muscle actin (1:1,000, Sigma) for arterial smooth muscle cells, M38 (undiluted, Developmental Studies Hybridoma Bank) for procollagen-synthesizing fibroblasts, and cytokeratin (1:100, Dako) for epicardial cells. The pan-leukocyte marker CD45 (1:100) was used to detect tissue macrophages in conjunction with QH1 staining as described (20). These second primary antibodies were detected with species-appropriate Cy2-coupled secondaries (1:100, Jackson Immuno) with a heat denaturation step included in case of mouse immunoglobulin G (IgG). All dilutions and rinses were with TBSA-BSAT. Control sections that were not stained (for tissue autofluorescence) or stained with secondary antibody only (for nonspecific binding of the secondary antibody) were also processed (3). The sections were then counterstained with DRAQ5 (1:1,000 in distilled water, BioStatus Ltd.) for nuclei, dehydrated with ascending ethanol series, cleared in xylene, and mounted in DEPEX mounting medium (Electron Microscopy Sciences).

Imaging was performed on an Olympus BH2 microscope fitted with a Canon G5 camera (visible staining) and Leica TCS SP2 AOBS confocal system (fluorescence). For presentation, images were adjusted (background subtraction, Unsharp Mask filtering) and labeled in Adobe Photoshop 8.0 (Adobe Systems).

Quantitative analysis

Numbers of quail cells present in the myocardium were counted on systematic random samples from left ventricular wall. Images were taken at 20× magnification of sections stained with DAB and either QH1 or QCPN. Counting was performed using a transparent overlay and pencil tool in Adobe Photoshop, and the number of dots placed (each representing a single quail cell) was then automatically counted using ImageJ (NIH freeware). The number per volume unit of the myocardium was then calculated after calibration, using the known field size (101,467 μm^2) and section thickness (10 μm).

The percentage of quail cells differentiating into cardiac lineages was enumerated on images of sections double stained for QCPN (a quail nuclear marker) and smooth muscle actin (coronary smooth muscle marker) or sarcomeric actin/myosin heavy chain (myocyte markers). Co-localization was evaluated on single optical sections taken with a 40 × 1.25 NA oil immersion objective (0.19 microns/pixel resolution) on systematic samples from left ventricular myocardium. The actual percentage was calculated by dividing the number of quail cells positive for the particular marker by the total number of quail cells in the field.

The frequency of fusion between chick and quail cells was estimated by counting 2,000 lacZ-positive cells from transgenic chick within whole-mount lacZ-stained quail myocardium, sectioned and then immunostained with either QCPN or QH1 quail marker. All suspicious double-labeled cells were photographed using a 100× oil immersion objective, and evaluated by three independent observers. Calculations were made by dividing the number of double-positive cells per total number of lacZ-positive cells counted.

RESULTS

Circulating stem cells are found in multiple organ systems

Analysis of serial sections from parabiotic chick embryos revealed abundant presence of quail cells in almost all organ systems. As expected, examples of engraftment and resulting colonies in the bone marrow were found (Fig. 1). There was also strong colonization of the spleen, thymus, and bursa of Fabricius, indicating lymphopoietic chimerism (Fig. 1), which is supported by finding of numerous cells in the submucosa of the gut (Fig. 2). In the liver, the only quail cells detected were lining the sinuses (Fig. 2), and some were also associated with smooth muscle liver capsule. High numbers of quail cells were found in the kidney (Fig. 3), where they localized both to the glomerular capillary walls and mesangium and to the peritubular interstitial space. Contribution to the peritubular capillary network was observed also in the gonad. Further organs showing colonization included the lungs (Fig. 4), skin (Fig. 5E), and the vascular components of the central nervous system. In spinal meninges, there was abundance of quail cells distributed around the spinal blood vessels as well as in the surrounding loose connective tissue. Few cells were also scattered throughout the spinal cord substance, but none of them showed typical neuronal morphology. The area with highest concentration within the brain was the choroid plexus (Fig. 4); however, no endothelial differentiation was observed.

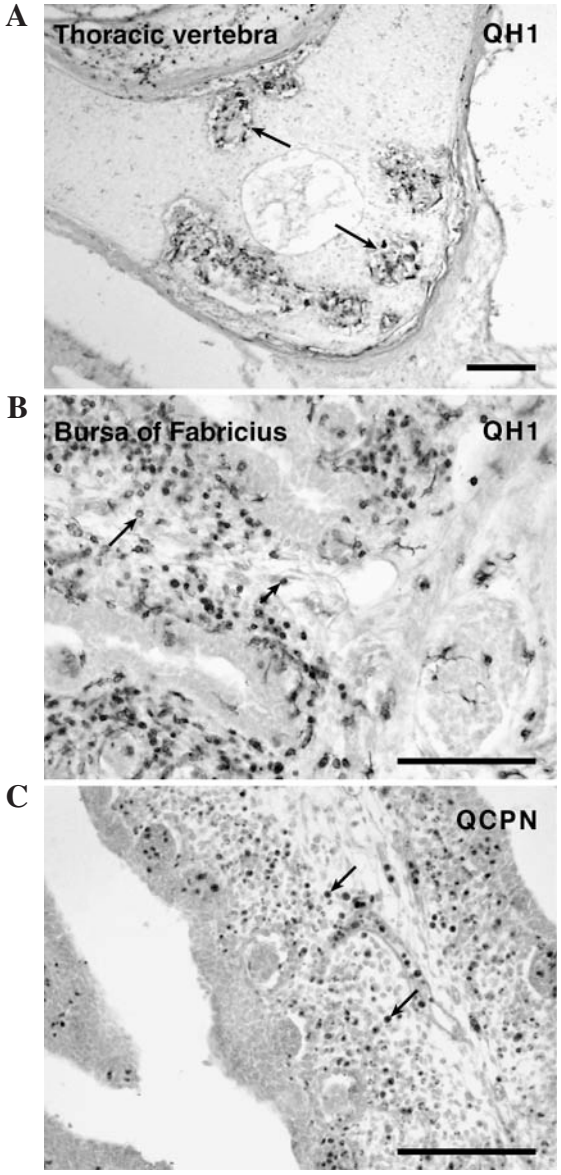


FIG. 1. Hematopoietic and lymphatic system engraftment. Parabiotic chick embryo sampled at ED15. (**A**) Colonies of quail-derived cells detected by QH1 antibody in chick bone marrow. (**B** and **C**) Numerous quail cells are found also in the lymphopoietic system. DAB staining; scale bars, 100 μm.

Contribution of circulating stem cells to developing vasculature

The distribution of circulation-derived cells suggested their contribution to the developing vasculature (Figs. 5 and 6). Detailed analysis showed their contribution to endothelial lineage in the liver and kidneys (Fig. 5A,B), but no such contribution was found in other organs. Differentiation in smooth muscle of arterial tunica media (Fig. 5C,D) was documented in the coronary arteries, and the frequency estimated by cell counting in three hearts was 15–20%. In addition, expression of the fibroblast marker procollagen was documented around skin arteries, and some periarterial circulation-derived cells expressed this marker, suggesting their differentiation to the adventitial layer (Fig. 5E). The majority of periarterial cells did not express any tissue-specific markers, and their morphology together with CD45 pan-leukocyte marker expression in some (Fig. 5F) led to conclusion that they are mostly tissue macrophages.

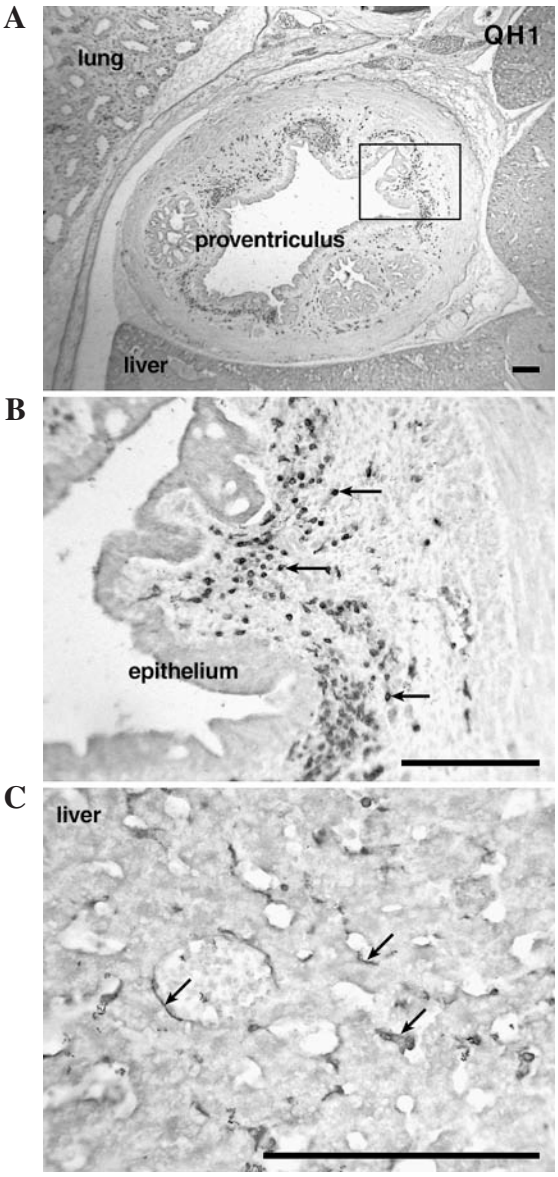


FIG. 2. Circulation-derived cells colonize the gastrointestinal system. (**A** and **B**) High concentration of QH1-positive cells is found in the submucosa. (**C**) In the liver, quail cells are occasionally found in the endothelial lining of the sinusoids, but there is no contribution to hepatocyte lineage. DAB staining; scale bars, 100 μm.

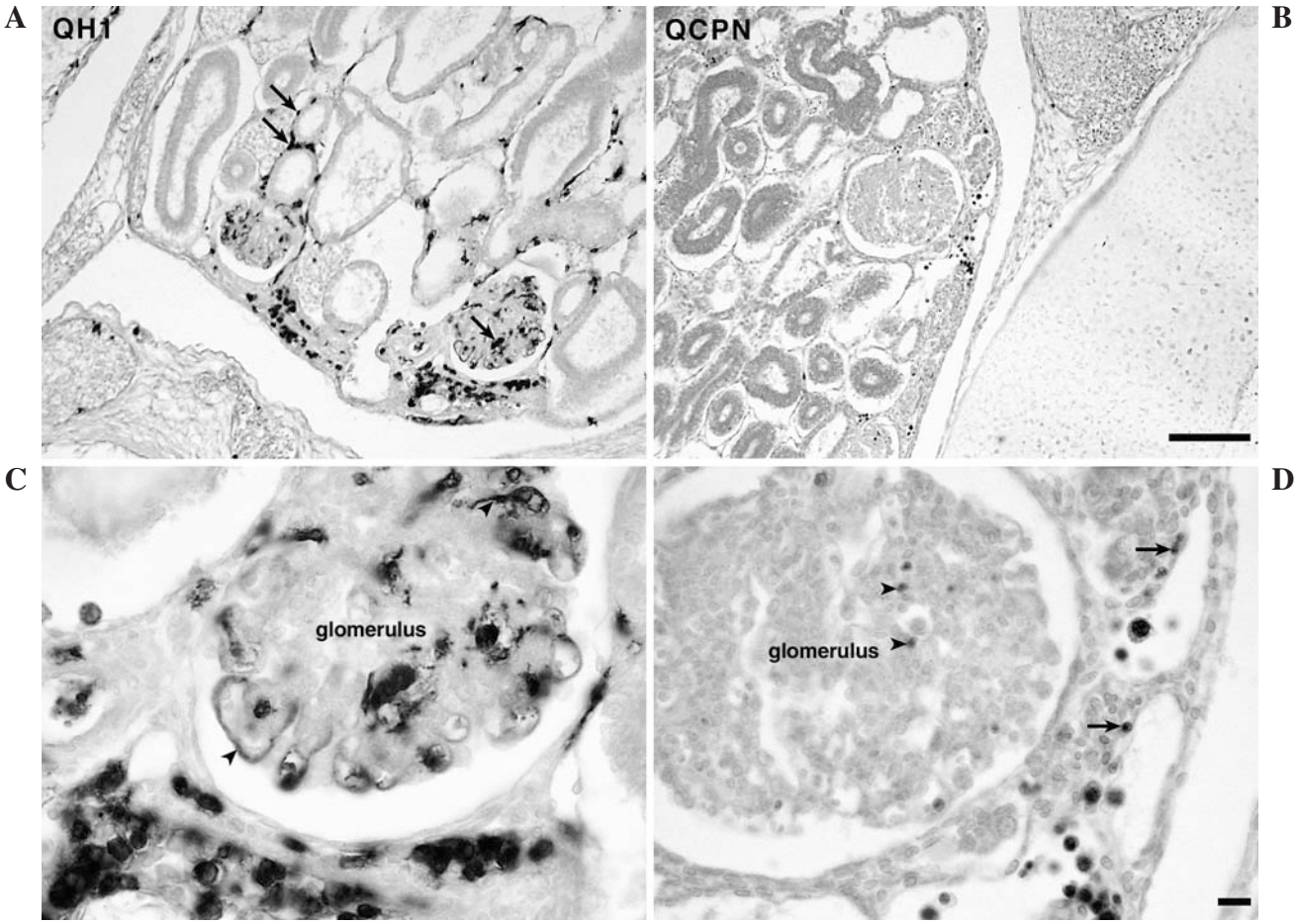


FIG. 3. Distribution of circulation-derived cells in the kidneys. (**A** and **B**) Low-power views show both QH1- and QCPN-positive cells in association with glomeruli and tubules. (**C** and **D**) Higher magnification shows that these cells are present in the mesangium; some exhibit typical flattened endothelial morphology (**C**, arrowhead) whereas other are present throughout interstitium (**D**). DAB staining; scale bars, 100 μm (**A,B**) and 10 μm (**C,D**).

Circulating stem cells differentiation in the heart

Within the heart, circulation-derived cells were found, in addition to myocardium, also in the developing valves and subepicardial tissue (Fig. 6). Counting of QCPN-positive cells within four separate hearts gave an average of 21,500 $\pm$ 10,200 (SD) cells per mm^3 of the left ventricular wall. On the basis of previous studies in the adult systems suggesting involvement of bone marrow stem cells in cardiac repair, a detailed analysis of their potential contribution to cardiomyogenic lineage was performed. Double immunolabeling with sarcomeric actin or myosin heavy chain (myocyte markers) showed that quail cells differentiated into cardiac myocytes (Fig. 6G,H). Quantitaive data obtained by counting percentage of quail cells expressing the cardiac markers in three different hearts showed that this occurred at a frequency of 5–40%. The majority of cells, however, did not express these markers, suggesting their contribution to cardiac fibroblasts, tissue macrophages (documented by their morphology and expression of CD45 surface marker, Fig. 6I) or yet undifferentiated state. Despite the lack of a suitable fibroblast marker in the chick at this stage, this notion was supported by expression of procollagen by a subset of arteriole-associated quail cells in other locations such as the skin (Fig. 5E).

Because there is evidence that some of the potential of the stem cells is a result of cell fusion rather than differentiation, parabiosis with LacZ transgenic chick was employed to obtain a second positive marker for investigating this possibility. Counting of 2,000 LacZ-positive cells within heart tissues of parabiotic quail counterstained with QH1 or QCPN markers (Fig. 6B,D) showed that in comparison with differentiation, cell fusion is a rare event, occurring with a frequency of two to three cells per 1,000.

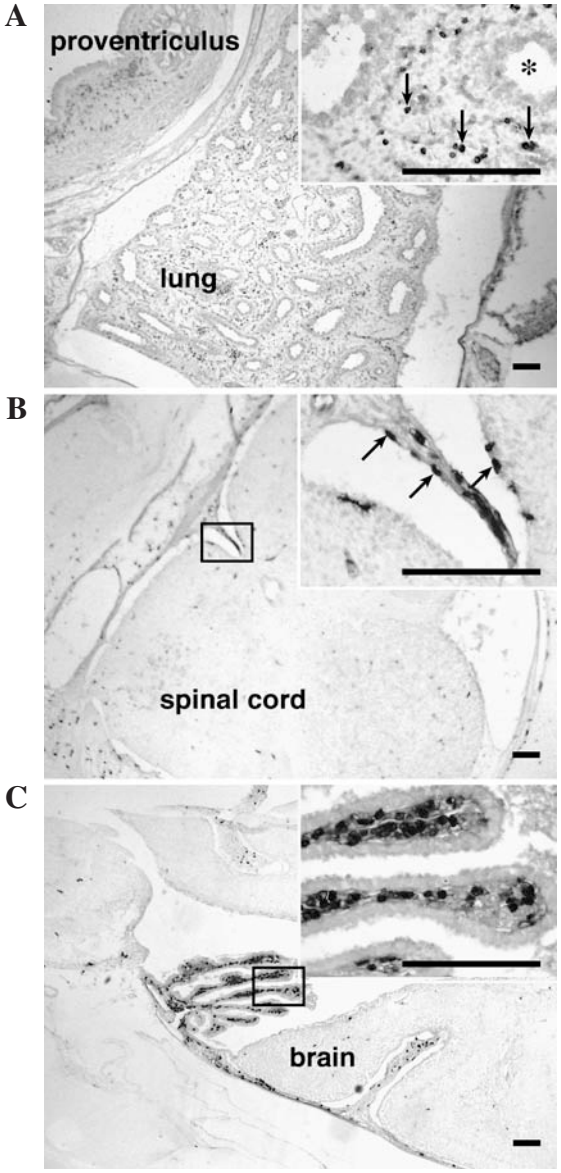


FIG. 4. Presence of circulation-derived cells in respiratory and central nervous system. (**A**) In the lung, quail cells are present throughout the parenchyma with clustering around developing vasculature (*inset*). (**B**) In the spinal cord, a high concentration of quail cells is present in the meninges (*inset*) as well as in the subarachnoid space. (**C**) In the brain, the highest concentration of quail cells is found in the vascular structures of the choroid plexus (*inset*). DAB staining; scale bars, 100 μm (10 μm for the insets).

Intracardiac injection of bone marrow cells reveals their cardiogenic potential

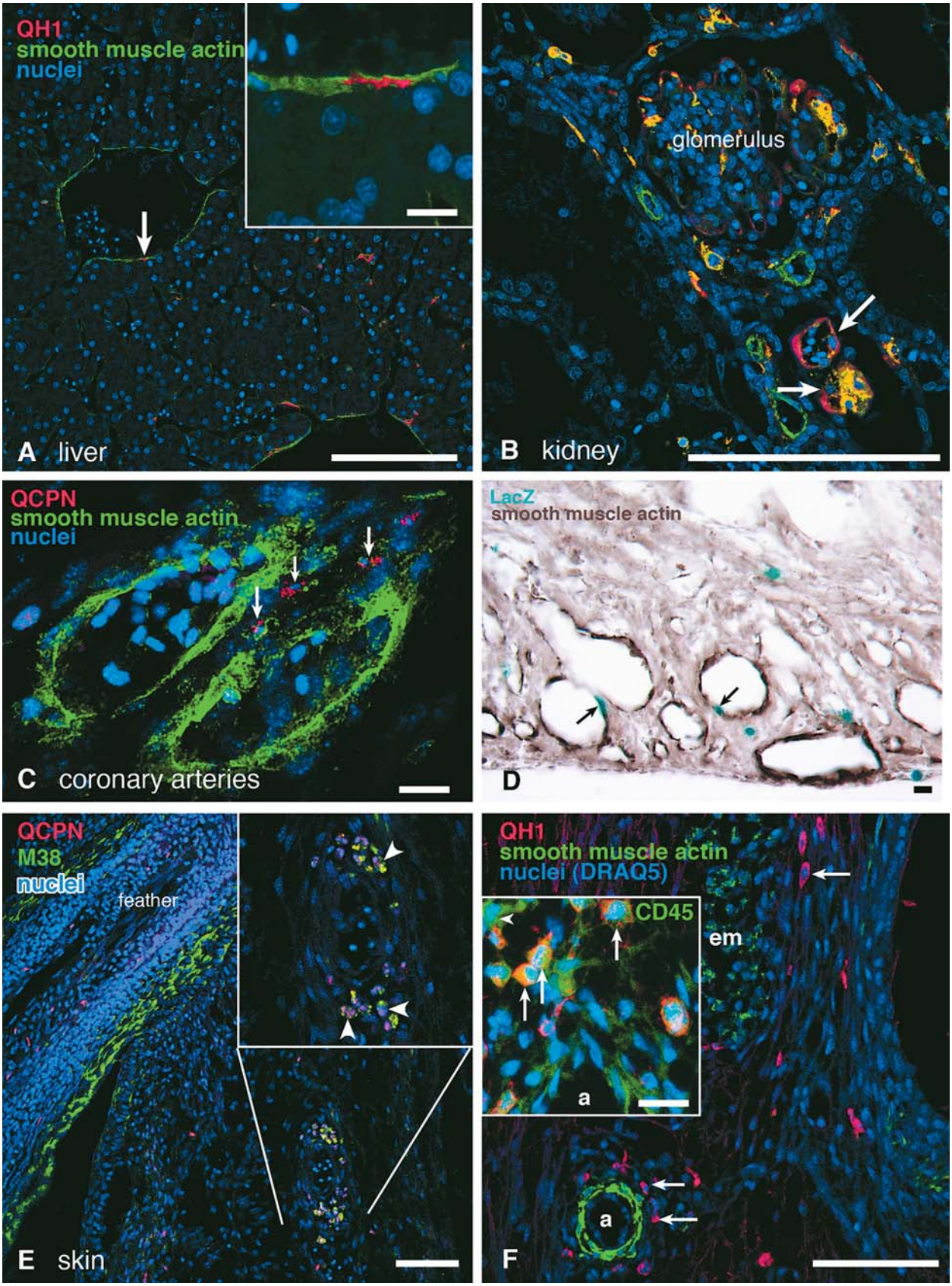
To verify further the cardiogenic potential of bone marrow cells, which are the progenitors of most of the cells in the circulation, in vivo, we performed injection of freshly isolated quail bone marrow into ED8 chick hearts. Sampling at 10 days later (ED18) showed the persistence of the grafted cells within the injected area (Fig. 7). Probing for their cardiogenic differentiation showed that subpopulations of these cells were expressing smooth muscle actin (coronary smooth muscle marker, 25%), cytokeratin (epicardial marker, $<$1%), as well as sarcomeric actin (myocyte marker, 8%; Fig. 7E–G).

DISCUSSION

Most studies to date on the plasticity of HSC potential have examined the adult, focusing mostly on their potential to repair tissue under pathological conditions. This has led to a contentious debate among opponents and proponents of HSC plasticity (3, 5, 9, 10, 21). The issue of blood stem cell plasticity in the embryo usually elicits a more narrow discussion, because the main point of contention has been whether the embryo possesses cells sharing both blood and endothelial (i.e., hemangioblastic) cell potential (20,22–24). This study on the physiological role of circulating cells, most of which are of hematopoietic origin, during embryogenesis may provide novel insights on the phenotypic capabilities of HSCs. The well-established model system of embryonic quail/chick chimeras was employed in our fate mapping studies of circulating cells. Our findings indicate that blood-borne stem cells do in fact possess a broad potential and contribute to the development of various tissues in the embryo.

Mosaic embryos produced by chick/quail parabiosis produced levels of chimerism in the bone marrow, spleen, thymus, and bursa that are consistent with previous studies (23,25). There was some variability in the extent of colonization, which correlated with observed mixing of the blood based on presence of quail cells in chick embryonic blood, and was probably due to the degree of anastomosis between the two circulations and time point of its establishment. In addition, there was a substantial number of cells localized to other areas where immune

FIG. 5. Contribution of circulation-derived cells to vasculature. (**A** and **B**) Circulating cells (arrows) contribute to endothelium in the liver and kidney. Scale bars, 100 μm or 10 μm (*inset*). (**C** and **D**) Differentiation into arterial smooth muscle is documented by co-localization of nuclear marker with smooth muscle actin expression detected by double immunohistochemistry (**C**) or whole-mount LacZ staining (**D**). Double-positive cells are indicated by the arrows. Scale bars, 10 μm. (**E**) Double immunohistochemistry with anti-procollagen (M38) antibody shows differentiation of some periarterial cells into adventitial fibroblasts (inset, arrowheads). (**F**) QH1-positive cells (arrows) are associated with arteries and smooth muscles (erectors of the feathers, em). (*Inset*) Numerous perivascular cells co-express QH1 with pan-leukocyte CD45 surface marker. A CD45-positive cell of chick origin is indicated by arrowhead. Scale bars, 50 μm or 10 μm (*inset*).


QH1
smooth muscle actin
nuclei
A liver
glomerulus
B kidney
QCPN
smooth muscle actin
nuclei
C coronary arteries
LacZ
smooth muscle actin
D
QCPN
M38
nuclei
feather
E skin
QH1
smooth muscle actin
nuclei (DRAQ5)
CD45
em
a
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F

FIG. 5.

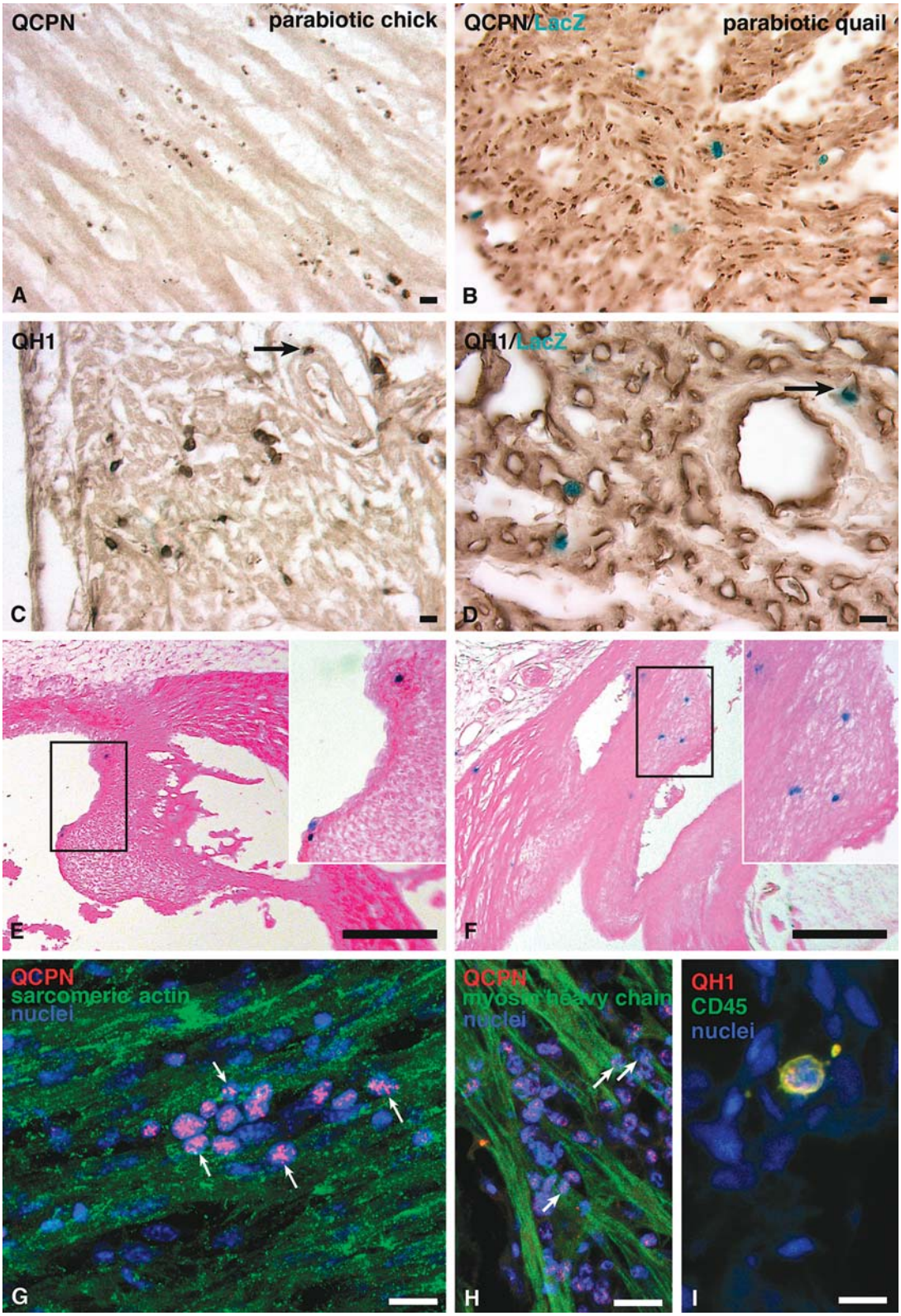

FIG. 6. Circulation-derived cells in the heart. (**A** and **C**) Detection of quail cells in the ED15 parabiotic chick heart using two different quail-specific markers. (**B, D, E, F**) Whole-mount LacZ-stained and sectioned quail heart parabiotic with LacZ-transgenic chick. Blue cells of chick origin are present in the same general location (e.g., periarterial, arrow in **D**–compare with **C**, or in the valves in **E** and **F**). Hematoxylin & Eosin staining (**E,F**), DAB staining (**A–D**); scale bars 10 μm (**A–D**) or 100 μm (**E,F**). (**G** and **H**) Differentiation of quail cells (labeled with nuclear marker QCPN) into cardiomyocytes using sarcomeric actin and myosin, respectively. Scale bars, 10 μm. (**I**) Intramyocardial macrophage, double labeled with QH1 (quail surface marker) and CD45 (pan-leukocyte marker). Scale bar, 5 μm.

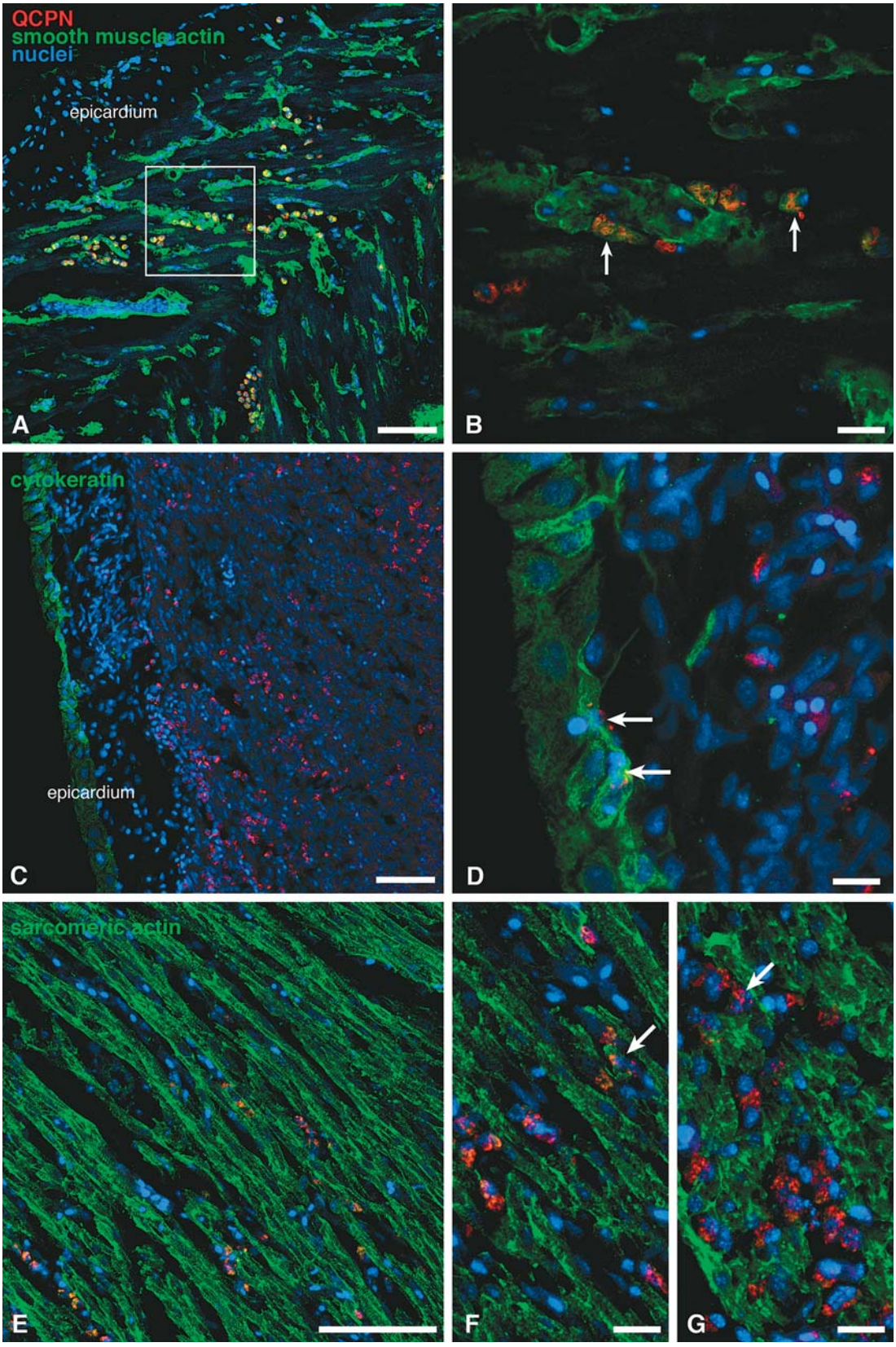

FIG. 7. Differentiation of injected bone marrow cells. (**A** and **B**) In a subset of cells, expression of smooth muscle actin is detected (arrows). (**C** and **D**) Some cells located in the epicardium express the cytokeratin marker (arrows). (**E–G**) Throughout the myocardium, examples of cells expressing sarcomeric actin (myocyte marker; arrows) can be found. Note that these cells show rather circular, immature morphology characteristic of newly differentiated myocytes. Scale bars, 50 μm (**A,C,E**) and 10 μm (**B,D,F,G**).

cells are known to be present, such as the skin (Langerhans cells), the submucosa of the gut, and the perialveolar space in the lung (macrophages, or coniophages). Their frequent association with developing arteries, and evidence of differentiation of a subset of circulation-derived cells into smooth muscle and adventitial fibroblasts, is in agreement with studies in the adult mammalian systems, where contribution of injected bone marrow stem cells to coronary vessels was demonstrated (3). In the kidney, association of circulation-derived cells with the glomerulus is in agreement with recently described origin of the mesangial cells from the bone marrow (26). Localization QH1-positive cells around the tubules is consistent with their contribution to abundant peritubular plexuses, and endothelial differentiation was also confirmed by transmission electron microscopy (our unpublished data, 2005) using established morphological nuclear markers (25). Bone marrow stem cells (both "stromal-mesenchymal" and "hematopoietic") are involved in regeneration of renal vascular and tubular parenchyma after ischemic injury (27). Because of their presence in normal kidneys, it is likely that these stem cells modulate normal renal function directly and/or via paracrine effects. Alternatively, they may represent the remnants of transitory HSC population described in the aortico-gonadal region (23), explaining the abundance observed in the developing gonad. The exact role of circulating stem cells in normal renal development is not fully understood.

Both the developing liver and brain were extensively examined for evidence of circulating cell contributions, because HSCs have been reported to possess the capacity to differentiate into hepatocytes or neuronal cells in the adult. However, no quail-derived hepatocytes were detected, because the only cells of quail origin found in chick liver were endothelial cells and those of macrophage morphology associated with intrahepatic vasculature and liver capsule. Likewise, no evidence of neuronal differentiation of circulating cells was observed. In a similar study focused on angiogenesis in the central nervous system (20), abundant cells of quail origin were found in perimeningeal vessels, but no invasion of the neuroepithelium proper was documented. In agreement with our data, no endothelial differentiation was reported in brain vessels by these authors. Although our inability to confirm results from the adult models may reflect intrinsic differences in circulating cell contributions in the embryo and adult, this disparity may be due to other factors, such as time interval, mode of delivery, and model system used. In this respect, modification of the parabiosis setup, according to originally published technique involving joining two eggs at 8 days of incubation (28), that allows post-hatching survival might provide insight into postnatal plasticity of circulating stem cells in birds.

Chimeric embryos consistently displayed measurable numbers of quail cells residing in the chick heart, including many that appeared to differentiate into cardiac myocytes. The range was wide within both the individual hearts and embryos, suggesting a clonal origin of quail cell clusters from a single cell, and possibly also different potentials of such cells. Of note, the morphology of most of these myocytes was reminiscent of immature myocytes derived from the adult bone marrow or resident cardiac stem cells (3,29), i.e., rounded with a thin layer of actin- or myosin-positive cytoplasm. On the basis of the average number of quail-derived cells exhibiting cardiomyocyte markers within each stained section, and the total number of quail cells per heart, it could be estimated that circulating cells may contribute as many as 0.9% of cardiomyocytes at this developmental stage. Furthermore, $<0.3\%$ of circulation-derived cells within the heart displayed both quail and chick markers, indicating that fusion under physiological conditions is a rare event.

Yet, the contribution of circulating cells to the adult myocardium has been a controversial topic. The magnitude of such an event in the adult systems seems to be highly variable, being reported as highly significant by some groups (3–5) as well as questioned or regarded as a product of cell fusion by others (9,10,30). Our results would appear to lend credence to the proposition that stem cells associated with the blood have myocardial potential. Apart from myocytes and vasculature, the quail cells were found in the cardiac interstitium of the chick hearts. A proportion of circulation-derived cells might contribute to cardiac fibroblasts, although no definitive marker is available at this stage of development. This would also explain the finding of circulation-derived cells in the developing valves and in the subepicardium. Finding that some of the perivascular cells were macrophages raises the question of possible hematopoietic origin of resident cardiac stem cells, because no non-myocytes are present in the wall of early tubular heart and several extracardiac contributions (epicardium, neural crest) were uncovered relatively recently (31,32). Unfortunately, this possibility could not be verified immunohistochemically because the established stem cell markers (sca-1, c-kit) did not react with avian tissues. Injection of crude quail fetal bone marrow showed persistence of the graft, expanding on our preliminary observations, which showed active proliferation of the injected cells after 48 h (33). At a 10-day sampling interval, injected cells were found differentiating into all major cardiac lineages (myocytes, coronary vasculature, epicardium; Fig. 7), recapitulating the results from the parabiosis experiments. It appears that part of this effect is due to local environment, because these cell fates do not normally occur inside the bone marrow. Characterization of environmental cues, together with purification of the source cell population, should provide optimized cell sources for cardiac regen-

erative therapy of congential malformations such as a hypoplastic left ventricle.

In conclusion, we have shown that circulating cells contribute to multiple organ systems during prenatal development and differentiate into cardiac and vascular cell types. The data presented here rule out cell fusion as the mechanism that accounts for this phenotypic plasticity of circulating cells. A possible mechanism that has not been ruled out by the present study is that the xenografts in the chimeric animals produced local areas of inflammation and transdifferentiation of immune response cells. However, because obvious signs of inflammation were not observed, most notably cell death revealed by lack of anti-caspase3 immunostaining (data not shown), it is likely that the results reported here are due to the differentiation of circulating stem cells to non-blood cell types. Future experimentation employing parabiosis of recently developed chickens transgenic for LacZ (17) or green fluorescent protein (GFP) (34) in a turkey shell (35) or two-egg parabiosis setup (28) should provide the definite answer. One implication of the present study is that the wide-ranging tissue potentiality of circulating stem cells observed in the embryo is a normal property of these cells, which accounts for the reported phenotypic plasticity of adult hematopoietic stem cells. Future studies examining the factors influencing the physiological processes that direct the phenotypic capacity of these circulating embryonic stem cells may hold great promise for novel prenatal cell-based therapeutic strategies.

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