RAPID COMMUNICATION

Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon

M Brittan, T Hunt, R Jeffery, R Poulsom, S J Forbes, K Hodivala-Dilke, J Goldman, M R Alison, N A Wright

Gut 2002;**50**:752–757

See end of article for authors' affiliations

Correspondence to: M Brittan, Histopathology Unit, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX, UK; mairi.brittan@cancer.org.uk

Accepted for publication 14 February 2002

Background and aims: In order to establish whether extraintestinal cells contribute to the turnover and repair of gastrointestinal tissues, we studied the colons and small intestines of female mice that had received a male bone marrow transplant, together with gastrointestinal biopsies from female patients that had developed graft versus host disease after receiving a bone marrow transplant from male donors.

Methods: Using in situ hybridisation to detect Y chromosomes and immunohistochemistry, we demonstrated that cells derived from injected bone marrow frequently engrafted into the intestine and differentiated into pericryptal myofibroblasts.

Results: In the human intestine, we confirmed by combining in situ hybridisation with immunostaining for smooth muscle actin that the bone marrow derived cells within the intestine exhibited a myofibroblast phenotype. In female mouse recipients of male bone marrow grafts, we observed colocalisation of Y chromosomes and clusters of newly formed marrow derived myofibroblasts. While few of these were present at seven days after bone marrow transplantation, they were numerous at 14 days, and by six weeks entire columns of pericryptal myofibroblasts could be seen running up the sides of crypts in both the small intestine and colon. These columns appeared to extend into the villi in the small intestine. Within the intestinal lamina propria, these Y chromosome positive cells were negative for the mouse macrophage marker F4/80 antigen and CD34.

Conclusions: Bone marrow derived pericryptal myofibroblasts were present in the mouse intestine following irradiation and bone marrow transplant, and in the intestines of human patients suffering graft versus host disease following a bone marrow transplant. Our data indicate that bone marrow cells contribute to the regeneration of intestinal myofibroblasts and epithelium after damage, and we suggest that this could be exploited therapeutically.

There has been excitement generated by the discovery that the stem cells in several tissues and organs are not as lineage restricted as was previously thought. Adult bone marrow cells show remarkable plasticity and can differentiate into hepatocytes[1–5] biliary epithelial cells,[4] endothelial cells,[6 7] skeletal muscle fibres,[8] cardiomyocytes,[9] neural cells,[10] and renal tubular epithelial cells.[11] These pathways can be bidirectional, with muscle and neuronal stem cells demonstrating the capability to form bone marrow.[12 13] Furthermore, cells that are apparently fully differentiated can transdifferentiate into other differentiated cells without cell division; for example, exocrine pancreatic cells can differentiate into hepatocytes.[14]

Intestinal subepithelial myofibroblasts (ISEMFs) are found in the lamina propria of the intestine under the epithelial cells, and are thought to exist as a syncytium. They are disposed in a fenestrated sheath in tight apposition to the intestinal crypts and extend through the lamina propria of the gut to merge with the pericytes of the blood vessels, contacting each other through adherens and gap junctions. ISEMFs appear in the human embryo at the 21st week of gestation, and it has been suggested that their origin might be from the neural crest or locally from mesenchymal stem cells, possibly sited in the muscularis mucosae.[15] There is some evidence that ISEMFs, like epithelial cells, proliferate and migrate alongside the crypt and, in the small intestine, along the villus axis, differentiating as they do from a discoid format at the crypt base to a stellate morphology at the apex, undergoing apoptosis at the tip.[16] Other studies indicate that they migrate slowly alongside the crypt, and then move off into the lamina propria to undergo polyploidisation.[17] Most workers agree that subepithelial myofibroblasts are of critical importance in epithelial:mesenchymal interactions, and that there is significant paracrine cross talk during epithelial differentiation. For example, several growth factors, such as hepatocyte growth factor, keratinocyte growth factor, and transforming growth factor β are secreted by the myofibroblasts, with receptors for these located on epithelial cells.[16]

We now provide further evidence for the plasticity of adult bone marrow cells by showing that they can differentiate into pericryptal myofibroblasts in both mice and humans. This discovery presents a new avenue for gene therapy to the gut whereby extensive and sustained engraftment of cells deep within the mucosae can be accomplished.

MATERIALS AND METHODS

Animals

All animal procedures were carried out under British Home Office procedural and ethical guidelines. Six week old female recipient mice (129Sv/C57B16) underwent whole body gamma irradiation with 12 Gray in a divided dose, three hours apart, to ablate their bone marrow, followed immediately by tail vein injection of male wild-type whole bone marrow (three male C57/black donor mice supplying bone marrow for 10 recipient female mice). Mice were housed in sterile conditions for six weeks. Animals were killed at seven days, 14 days,

Abbreviations: ISEMF, intestinal subepithelial myofibroblast; PBS, phosphate buffered saline; αSMA, α smooth muscle actin; PDGF, platelet derived growth factor; SSC, standard saline citrate.

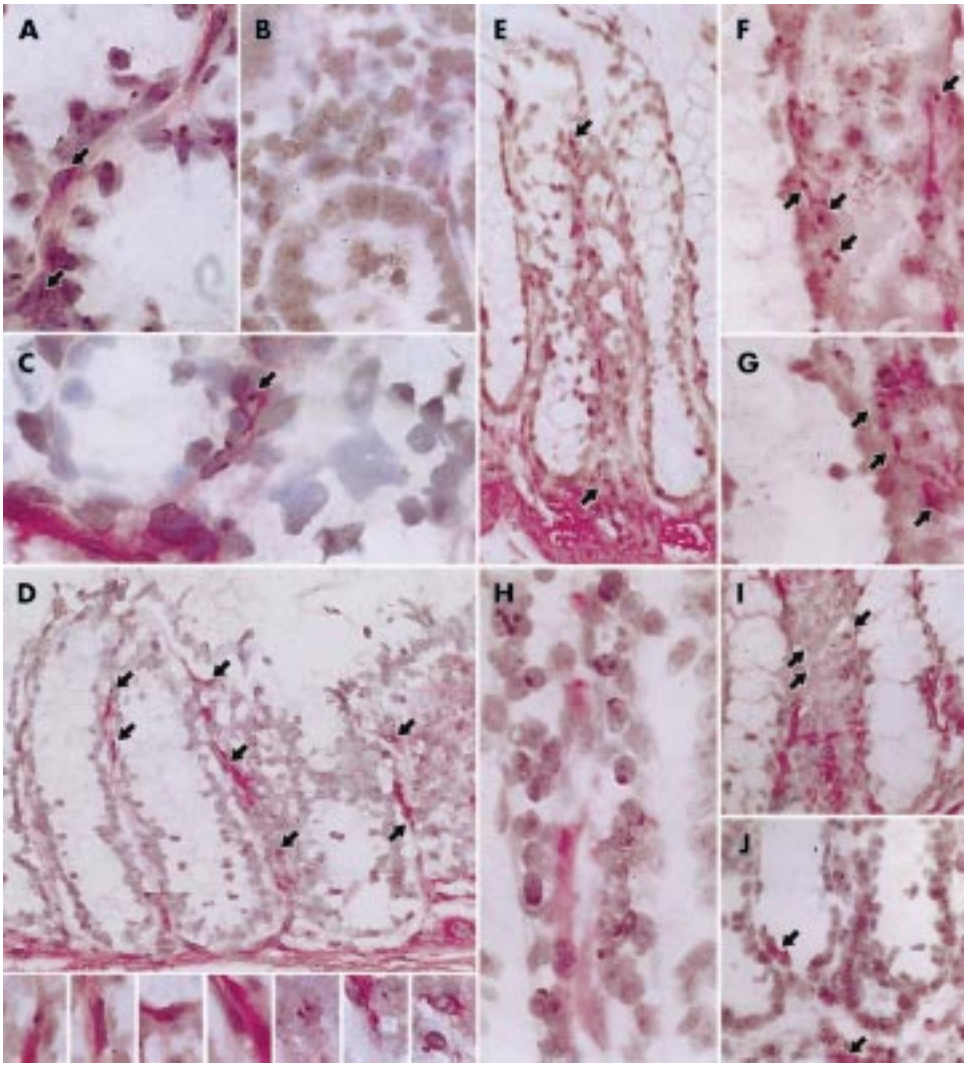

Figure 1 Y chromosome detection in the mouse. The Y chromosome is seen as a punctate brown/black density in the cell nucleus. Red staining of the cell cytoplasm identifies positive immunohistochemical staining. (A) Male positive control showing Y chromosome detection in colonic pericryptal myofibroblast cells immunostained with the α smooth muscle actin (αSMA) antibody. (B) Female control demonstrating lack of Y chromosome detection within colonic pericryptal myofibroblasts immunostained with the αSMA antibody. (C–G) Female mouse after male whole bone marrow transplantation showing Y chromosome positive myofibroblasts immunostained with the αSMA antibody. (C) Few αSMA immunoreactive Y chromosome positive cells present in the mouse colon seven days after male bone marrow transplant. (D) Numerous Y chromosome positive myofibroblasts (for example, arrows, see also inserts for high power views) immunoreactive for αSMA in female mouse colon 14 days after male bone marrow transplant. (E) Y chromosome positive myofibroblasts stained with αSMA in the colon at six weeks after transplant showing extension of the column right up to the top of the crypts. (F, G) High power view of Y chromosome positive myofibroblasts that are αSMA immunoreactive in female mouse colon six weeks after transplant. (H) Desmin positive cells within the lamina propria were not Y chromosome positive. (I) Y chromosome positive cells that are negative for the haematopoeitic progenitor marker CD34 in the mouse colon six weeks after transplant. (J) Y chromosome positive cells that are negative for the mouse macrophage marker F4/80 antigen (positively stained macrophages shown by arrows).

and six weeks following bone marrow transplant and their intestines were fixed in neutral buffered formalin before being embedded in paraffin wax.

Human material

Tissue from three female patients who had received unprocessed bone marrow transplants from male donors were analysed. These patients had subsequently had one or more small intestinal biopsies for suspected graft versus host disease. Biopsies were formalin fixed, routinely processed, and paraffin wax embedded. Male and female control intestinal tissues were obtained from the archives of Hammersmith Hospital, and all procedures were in accordance with local ethical guidelines.

Immunohistochemical analyses and Y chromosome detection

To identify the origin of pericryptal cells within the intestine, Y chromosome in situ hybridisation was combined with immunohistochemistry.

For mouse tissue we combined in situ hybridisation for the Y chromosome with immunohistochemistry for α smooth muscle actin (αSMA) (mouse monoclonal clone 1A4, A-2547; Sigma, St Louis, Missouri, USA). As further tests of the phenotype of bone marrow graft derived cells, we combined Y chromosome in situ hybridisation and immunohistochemistry for desmin (monoclonal antibody MDEII; Euro-Diagnostica, Arnhem, the Netherlands), a murine macrophage marker (rat polyclonal anti-mouse F4/80 antigen; MCAP497; Serotec, Kedlington, Oxford, UK) and the haematopoietic progenitor marker CD34 (rat polyclonal anti-mouse CD34; CL8927 AP; Cedarlane, Ontario, Canada).

To identify myofibroblasts in the human intestine sections, we immunostained for smooth muscle actin prior to in situ hybridisation for the Y chromosome.

Treatment of sections

Sections (4 μm) were dewaxed and incubated with 2.4 ml of 30% hydrogen peroxide in 400 ml methanol to block endogenous peroxidases, and taken through graded alcohols

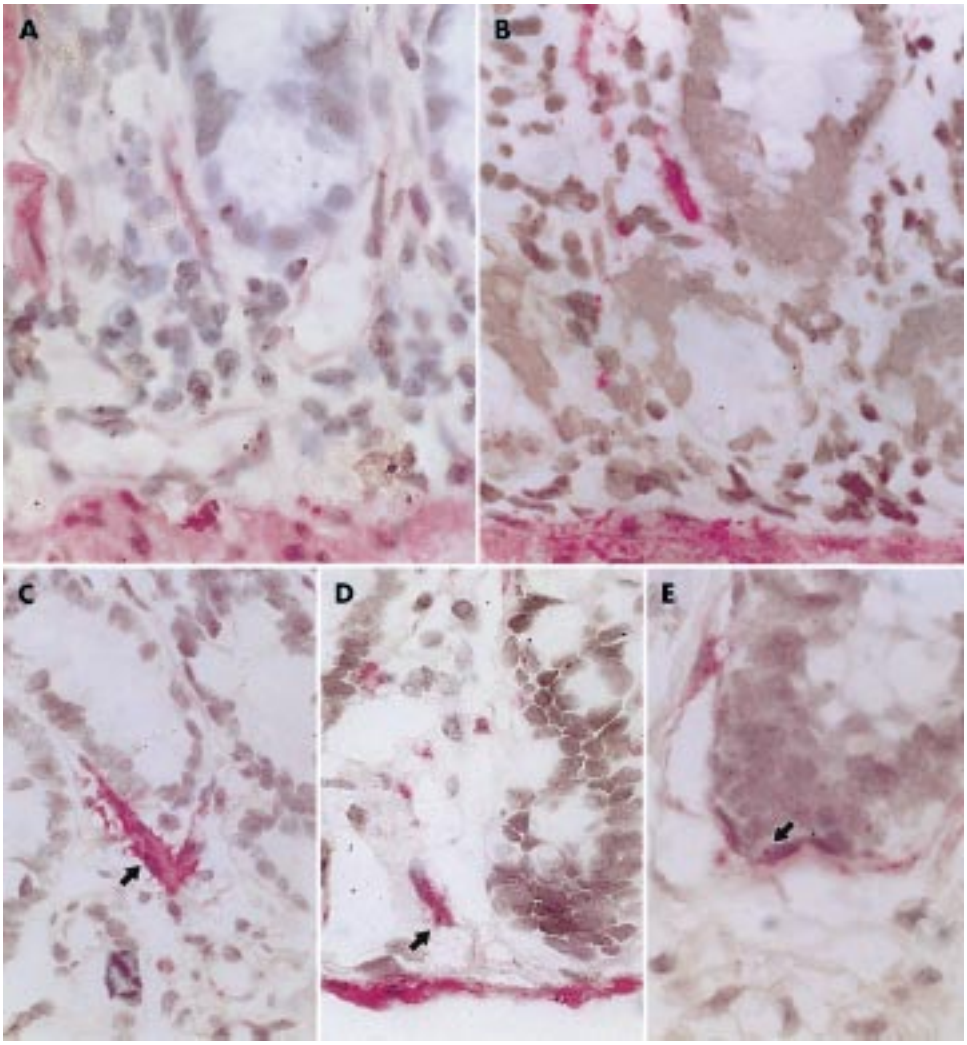

Figure 2 Y chromosome detection in the human small intestine. (A) Male control duodenum showing indirect immunodetection of the Y chromosome also immunostained for α smooth muscle actin (αSMA). (B) Female duodenal control showing absence of signal for the Y chromosome also immunostained for αSMA. (C–E) Human duodenal biopsies from female patients who received a bone marrow transplant from a male donor, showing myofibroblasts immunostained with αSMA and positive for the Y chromosome probe (arrows).

to phosphate buffered saline (PBS). Sections to be immunostained for F4/80 were incubated in bovine trypsin (Prod 390414M; BDH Laboratory Supplies, Poole Dorset, UK) (100 mg bovine trypsin, 100 mg $CaCl_2$, 100 ml distilled water, pH 7.8) at 37°C for 15 minutes and washed in PBS. Sections that were to be immunostained for desmin were microwaved in sodium citrate buffer (pH 6.0) for 10 minutes and washed in PBS to expose the antigen.

All tissues were incubated for three minutes in 20% acetic acid in methanol to block endogenous alkaline phosphatase. Slides were preincubated in normal rabbit serum (D0396; Dako, Ely, Cambridgeshire, UK) at 1/25 for 10 minutes. Primary antibodies were used at dilutions of 1/4000 for SMA, 1/20 for desmin, 1/50 for F4/80 antigen, and 1/100 for CD34; all sections were incubated for 35 minutes. The secondary antibody was biotinylated rabbit antimouse (E0354; Dako) for SMA and desmin diluted to 1/250, and rabbit anti-rat (BA-4001; Vector Laboratories, Orton Southgate, Peterborough, UK) for F4/80 and CD34 diluted to 1/100, both applied for 35 minutes. A tertiary antibody layer of streptavidin-alkaline phosphatase (D0396; Dako) diluted to 1/50 for 35 minutes followed this. Sections were washed in PBS between each antibody layer, and Vector Red substrate (SK 5100; Vector Laboratories) was applied for 15 minutes at room temperature. Sections were again washed in PBS prior to the in situ hybridisation protocol.

Human sections immunostained for SMA were treated to an identical protocol as the mouse sections. All sections were then taken through the in situ hybridisation protocol to detect the Y chromosomes.

In situ hybridisation

Sections were incubated in 1 M sodium thiocyanate for 10 minutes at 80°C, washed in PBS, and then digested in 0.4% w/v pepsin in 0.1 M HCl at 37°C for varying times depending on the pretreatment used for immunohistochemistry. The protease was quenched in 0.2% glycine in double concentration PBS and sections were then rinsed in PBS, post-fixed in 4% paraformaldehyde in PBS, dehydrated through graded alcohols, and air dried. For mouse tissues, an FITC labelled Y chromosome paint (Star-FISH, Cambio, Cambridge, UK, cat. No 1189-YMF-01) was used in the supplier's hybridisation mix. The probe mixture was added to the sections, sealed under glass with rubber cement, heated to 60°C for 10 minutes, and incubated overnight at 37°C. For the human probe, an FITC labelled Y chromosome paint (Star-FISH; cat. No 1083-YF-01) was used similarly, except the human slides were heated to 80°C for 10 minutes and incubated overnight at 37°C. All slides were washed in 50% formamide/2×standard saline citrate (SSC) at 37°C, washed with 2×SSC and then 4×SSC/0.05% Tween-20, and incubated with 4×SSC/0.05% Tween/5% milk powder for 10 minutes at 37°C. All slides were washed with PBS and incubated with 1/25 peroxidase conjugated antifluorescein antibody (150 U/ml, cat. No 1426346; Boehringer Mannheim, Mannheim, Germany) for 60 minutes

at room temperature. Slides were developed in 3,3'-diaminobenzidine plus hydrogen peroxide, counterstained with haematoxylin, and mounted in DPX.

RESULTS

We identified frequent Y chromosome containing cells within both the intestines of normal female mice that had received a male bone marrow transplant and human female patients who had received a bone marrow transplant from male donors.

Mouse studies

In the colons of male control mice, 69.2% of pericryptal myofibroblasts were positive for the Y chromosome (fig 1A) whereas there were no Y chromosome positive myofibroblasts in female control mice (fig 1B). The fact that the Y chromosome could not be detected in every myofibroblast in the male controls was expected because the histological sections were of finite thickness and the Y chromosome would not be present in all nuclear fragments. Accordingly, the cell counts in murine transplant tissues were divided by a factor of 0.692 to provide the likely percentages of Y chromosome positive cells in the female mice engrafted with male bone marrow.

Following transplantation of normal male bone marrow into normal but irradiated female mice, we observed frequent Y chromosome containing cells within the lamina propria of the colon and small intestine. In recipient female mice at seven days there were few pericryptal myofibroblasts with Y chromosomes (fig 1C); at 14 days after bone marrow transplantation up to 49.4% (adjusted count) of pericryptal cells contained a Y chromosome (fig 1D) which increased to 57.6% at six weeks after transplant. At six weeks there were many positive cells with columns of male myofibroblasts extending up the side of the crypt in both the colon and small intestine (fig 1E–G). In the small intestine, this column appeared to extend into the villus and indeed up to the villus tip.

Several lines of evidence support the contention that many of the Y chromosome containing cells in the female recipients were myofibroblast in nature: they had appropriate morphology, were strongly SMA positive (fig 1C–G), and negative for desmin (fig 1H). Some of the bone marrow derived cells were clearly negative for the mouse macrophage marker F4/80 and haematopoietic progenitor marker CD34 (fig 1I, J). Some Y chromosome positive cells were present in the female mouse colon that expressed F4/80 and CD34, although these are likely to be haematopoietic cells and macrophages present in the transplanted bone marrow that had not transdifferentiated.

Y chromosome positive myofibroblasts were seen extending from the pericryptal zone into the lamina propria, forming a network in animals at 14 days and six weeks (fig 1D, E), and these also extended to the adventitia of small arterioles in the submucosa.

Human studies

In male control intestinal biopsy material, pericryptal cells were positive for the Y chromosome (fig 2A). In the female intestinal control, no Y chromosomes were detected within pericryptal myofibroblasts (fig 2B).

In female patients who had received a bone marrow transplant from male donors (fig 2C–E), Y chromosome positive myofibroblasts (identified by SMA) were present in every patient although the frequency of these cells varied between patients. There was no distinct localisation of these cells in the villous tips or crypt bases, and their expression appears to be random. In this small series there was no obvious correlation between the degree of bone marrow engraftment and the time elapsed between bone marrow transplant and intestinal biopsy.

DISCUSSION

It is now very clear that adult stem cells exhibit remarkable plasticity: thus muscle and CNS derived stem cells can give rise to haematopoietic stem cells.[12 13] Bone marrow stem cells themselves can contribute to hepatocyte, biliary, endothelial, myogenic, glial, and renal lineages.[1–11]

Our present results show that an organised network of myofibroblasts, the pericryptal and lamina proprial meshwork, can receive a major contribution from the bone marrow. In our mouse studies there is no doubt that the marrow was the origin of the new ISEMFs, but in the human studies although donor bone marrow is the likely source of the pericryptal myofibroblasts, it cannot be unequivocally established due to lack of knowledge of the patient's genetic background.

While we believe that this is the first study to report that bone marrow stem cells can contribute to a normal organised system of myofibroblasts such as the ISEMFs, there have been several previous reports that have suggested that myofibroblasts can be derived from bone marrow. Thus Grimm and colleagues,[21] also analysing sex mismatched transplants in humans, demonstrated that some 30% of kidney myofibroblasts were of extrarenal origin. While bone marrow was not specifically documented as the origin of these cells, our own observations[11] strongly suggest that renal interstitial myofibroblasts can be so derived. Other observations have recorded that myofibroblasts will differentiate from human bone marrow mesenchymal stem cells when they are exposed to colorectal carcinoma cell lines in vitro,[22] and also when they are around foreign bodies introduced into the mouse peritoneal cavity.[23]

A further group of myofibroblasts in the intestine are the interstitial cells of Cajal. These cells are located close to mural neurones and act as pacemakers, propagating electrical events and modulating neurotransmission. They are said to be SMA positive and desmin positive, and are also immunopositive for c-kit and CD45.[19] We have identified Y chromosome positive cells in the muscularis layers of the female mouse intestine (data not shown), the phenotype of which will be determined by further immunohistochemical investigation.

Also included in the myofibroblast family are the stellate cells of the liver and pancreas.[20] Such cells express muscle and non-muscle genes, are contractile, and secrete a number of growth factors and cytokines, and of course synthesise extracellular matrix proteins.[19 20] Their proliferation is dependent on platelet derived growth factors (PDGFs) and their receptors: indeed PDGF-AA and PDGFR-α knockout mice display defective ISEMFs and profound gastrointestinal abnormalities.[24] An interesting possibility would be whether a bone marrow transplant might at least partially ameliorate these defects: Lagasse and colleagues[5] were able to correct the metabolic defect in fumaryl acetoacetate hydrolase–/– mice by such a procedure—the transplanted bone marrow stem cells transdifferentiated into functional hepatocytes.

Krause and colleagues[25] showed that a purified bone marrow stem cell population engrafted multiple organs, including lung and epithelial cells of the gut and skin. However, they did not report that the pericryptal sheath was so colonised; the absence of bone marrow stromal stem cells in the graft could account for this as we have transfused whole bone marrow. The origin of these engrafted cells could then be the stromal stem cells although while disputed, it is generally said that in humans transplanted stromal cells do not survive[26] and definitive proof must await the transplantation of defined populations of stromal and haematopoietic stem cells. However, it is interesting to speculate that the engrafted cells come from the population of circulating "fibrocytes" which, in the human, form between 0.1% and 0.5% of the non-red blood cell population[27] and which migrate to skin wounds in mouse and humans. These cells are certainly SMA positive in the wounds and express collagen I and III,

fibronectin, CD45RO, CD13, and CD34. It is possible that these cells derive from the transplanted male marrow cells, enter the circulation, and colonise the intestine.

Our observations indicate that bone marrow, albeit in circumstances surrounding a bone marrow transplant, can contribute to the turnover of the pericryptal myofibroblast sheath. Following irradiation, there is prominent loss of epithelial cells while pericryptal myofibroblasts disintegrate within 24 hours in the rat, as evidenced by loss of cadherin and SMA staining.[28] In humans, the pericryptal fibroblast count falls on a time scale very closely associated with that seen in epithelial cells, with only some immediate recovery occurring in fibroblasts, in contrast with the adjacent epithelial cells where full recovery seems to take place. Moreover, fibroblasts showed a gradually diminishing trend during the first year while the epithelial cell numbers appeared to be maintained.[29] Thus in both the mouse and human situation, bone marrow cells are apparently replacing cells killed by whole body irradiation.

However, in human graft versus host disease, while there was no doubt that myofibroblasts were derived from the transplanted bone marrow, these were far fewer than in the mouse. Moreover, we experienced considerable difficulty in accurately quantifying the number of Y positive cells in control human tissue and therefore calculation of a correction factor was inappropriate.

We observed lines of Y positive cells (fig 1D–G), highly suggestive evidence of clonal expansion, possibly with the bone marrow stem cells initially seeding the lamina propria near the base of the crypt and ISEMFs proliferating and migrating from here. There has been speculation that ISEMFs migrate *parri passu* with the crypt epithelial cells.[17] Our observations are consistent with bone marrow cells engrafting in the crypt base and migrating upwards into the upper crypt and villi, as Y chromosome positive myofibroblasts were present in the villi at two and six weeks but not at seven days. In the column of myofibroblasts that were positive for the Y chromosome, some did extend upwards into the villus; this might suggest that migration was continuous, although Neal and Potten[17] suggested that these cells moved laterally into the lamina propria at the top of the crypt, and became polyploid. We certainly did not see any SMA positive cells with multiple Y chromosomes, unlike the bone marrow derived polyploid hepatocytes we have encountered.[30] Alternatively, it may be simply that a number of cells entered locally or have occupied particular niches. In the patients studied here, there was wide variation in the proportions of myofibroblasts derived from extraintestinal sources; this may reflect differences in the degree of intestinal damage and also technical factors such as tissue fixation.

In the intestine, myofibroblasts form a continuous meshwork extending from the adventitia of the submucosal blood vessels through the lamina propria to the pericryptal sheath.[16] Our observations indicate that all of these cells can be derived from bone marrow.

The pericryptal myofibroblast sheath is closely associated with the epithelial cells of its crypt and is thought to influence epithelial behaviour by producing a number of growth factors. Stem cells within many different tissues are thought to reside within "niches" or groups of cells and extracellular substrates which provide an optimal microenvironment for stem cells to give rise to their differentiated progeny. ISEMFs surround the base of each crypt, a commonly proposed location for the intestinal stem cell niche, and it is well documented that ISEMFs influence epithelial cell proliferation and regeneration through epithelial:mesenchymal cross talk, and ultimately determine epithelial cell fate. Our observations suggest that bone marrow cells could be used as vehicles for gene delivery to the gut, to modify both myofibroblast and epithelial cell behaviour.

ACKNOWLEDGEMENTS

This work was supported by the Imperial Cancer Research Fund, now Cancer Research UK

Authors' affiliations

M Brittan, T Hunt, R Jeffery, R Poulsom, Histopathology Unit, Cancer Research UK, London, UK
S J Forbes, Histopathology Unit, Cancer Research UK, London, UK, and Department of Medicine, Faculty of Medicine, Imperial College of Science, Technology and Medicine (ICSTM), St Mary's Hospital, London, UK
K Hodivala-Dilke, Metastasis Laboratory, Cancer Research UK, St Thomas' Hospital, London, UK
J Goldman, Faculty of Medicine, Imperial College of Science, Technology and Medicine, St Mary's Hospital, London, UK
M R Alison, Histopathology Unit, Cancer Research UK, London, UK, and Department of Histopathology, ICSTM, Hammersmith Hospital, London, UK
N A Wright, Histopathology Unit, Cancer Research UK, London, UK, and Department of Histopathology, Barts and the London, Queen Mary's School of Medicine and Dentistry, London, UK

REFERENCES

1 **Petersen BE**, Bowen WC, Patrene KD, *et al*. Bone marrow as a potential source of hepatic oval cells. *Science* 1999;**284**:1168–70.
2 **Theise ND**, Badve S, Saxena R, *et al*. Derivation of hepatocytes from bone marrow cells in mice after radiation induced myeloablation. *Hepatology* 2000;**31**:235–40.
3 **Alison MR**, Poulsom R, Jeffery R, *et al*. Hepatocytes from non-hepatic adult stem cells. *Nature* 2000;**406**:257.
4 **Theise ND**, Nimmakalu M, Gardner R, *et al*. Liver from bone marrow in humans. *Hepatology* 2000;**32**:11–16.
5 **Lagasse E**, Connors H, Al-Dhalimy M, *et al*. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 2000;**6**:1229–34.
6 **Lagaaij EL**, Cramer-Knijnenburg GF, van Kemenade FJ, *et al*. Endothelial cell chimerism after renal transplantation and vascular rejection. *Lancet* 2001;**357**:33–7.
7 **Gao Z**, McAlister VC, Williams GM. Repopulation of liver endothelium by bone-marrow-derived cells. *Lancet* 2001;**357**:932–3.
8 **Ferrari G**, Cusella-De Angelis G, Coletta M, *et al*. Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;**279**:1528–30.
9 **Orlic D**, Kajstura J, Chimenti S, *et al*. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;**410**:701–5.
10 **Eglitis MA**, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 1997;**94**:4080–5.
11 **Poulsom R**, Forbes SJ, Hodivala-Dilke K, *et al*. Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 2001;**195**:229–35.
12 **Jackson KA**, Mi T, Goodell MA. Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci USA* 1999; **96**:14482–6.
13 **Bjornson CRR**, Rietze RL, Reynolds BA, *et al*. Turning brain into blood: A hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science* 1999;**283**:534–7.
14 **Shen CN**, Slack JM, Tosh D. Molecular basis of transdifferentiation of pancreas to liver. *Nat Cell Biol* 2000;**12**:879–87.
15 **Okada H**, Inoue T, Suzuki H, *et al*. Epithelial-mesenchymal transformation of renal tubular epithelial cells in vitro and in vivo. *Nephrol Dial Transplant* 1991;**15**(suppl 6):44–6.
16 **Powell DW**, Mifflin RC, Valentich JD, *et al*. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. *Am J Physiol* 1999;**277C**:183–201.
17 **Pascal RR**, Kaye GI, Lane N. Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. I. Autoradiographic studies of normal rabbit colon. *Gastroenterology* 1968;**54**:835–51.
18 **Neal JV**, Potten CS. Description and basic cell kinetics of the murine pericryptal fibroblast sheath. *Gut* 1981;**22**:19–24.
19 **Powell DW**, Mifflin RC, Valentich JD, *et al*. Myofibroblasts. I. Paracrine cells important in health and disease. *Am J Physiol* 1999;**277**:C1–9.
20 **Walker GA**, Guerrero IA, Leinwand LA. Myofibroblasts: molecular crossdressers. *Curr Top Dev Biol* 2001;**51**:91–107.
21 **Grimm PC**, Nickerson P, Jeffery J, *et al*. Neointimal and tubulointerstitial infiltration by recipient mesenchymal cells in chronic renal-allograft rejection. *N Engl J Med* 2001;**345**:93–7.
22 **Emura M**, Ochiai A, Horino M, *et al*. Development of myofibroblasts from human bone marrow mesenchymal stem cells cocultured with human colon carcinoma cells and TGF beta 1. *In Vitro Cell Dev Biol Anim* 2000;**36**:77–80.
23 **Campbell JH**, Efendy JL, Han C, *et al*. Haemopoietic origin of myofibroblasts formed in the peritoneal cavity in response to a foreign body. *J Vasc Res* 2000;**37**:364–71.
24 **Karlsson L**, Lindahl P, Heath JK, *et al*. Abnormal gastrointestinal development in PDGF-A and PDGFR-(alpha) deficient mice implicates a

novel mesenchymal structure with putative instructive properties in villus morphogenesis. *Development* 2000;**127**:3457–66.
25 **Krause DS**, Theise ND, Collector MI, *et al.* Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001;**105**: 369–77.
26 **Keating A**, Singer JW, Killen PD, *et al.* Donor origin of the in vitro haematopoietic microenvironment after marrow transplantation in man. *Nature* 1982;**298**:280–3.
27 **Abe R**, Donnelly SC, Peng T, *et al.* Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol* 2001;**166**:7556–62.
28 **Thiagarajah JR**, Gourmelon P, Griffiths NM, *et al.* Radiation induced cytochrome c release causes loss of rat colonic fluid absorption by damage to crypts and pericryptal myofibroblasts. *Gut* 2000;**47**:675–84.
29 **Wiernik G**, Perrins D. The radiosensitivity of a mesenchymal tissue. The pericryptal fibroblast sheath in the human rectal mucosa. *Br J Radiol* 1975;**48**:382–93.
30 **Forbes SJ**, Hodivala-Dilke KM, Jeffery R *et al.* Hepatocytes derived from bone marrow stem cells demonstrate polyploidisation. *J Hepatol* 2001;**34**(suppl 1):20–1.