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Leading article

Transplantation of cultured small bowel enterocytes

Development of successful primary culture techniques for mammalian cells, have permitted the study of basic molecular mechanisms governing physiological and pathological cellular processes.[1–4] In a therapeutic context, appropriate cells have been successfully isolated, maintained or cultured in vitro and subsequently transplanted to restore bone marrow function in haemopoetic disorders,[5–7] to provide skin cover in major burns[8–11] and insulin independence after pancreatic islet transplantation.[12] The use of cultured osteoblasts to enhance bone graft function[13] and hepatocyte transplantation in acute hepatic failure[14–16] are currently being evaluated.

Central to the application of these techniques in renewal tissues has been the isolation and maintenance ex vivo of progenitor or pluripotent stem cells, which by definition have the capacity for both self maintenance and for production of distinct functional cell lineages. Comprehension of stem cell biology of specific tissues has aided development of the above methods and may be appropriate for development of similar techniques, for the small bowel.

Stem cells of the small intestine

The small bowel mucosa has distinct proliferative and functional epithelial cell subpopulations. Stem cells, which are few in number[17–19] and intermediate transit cells, which have limited proliferative capacity, occupy the lower two thirds of each crypt.[20] Stem cells divide, giving rise to both new stem cells and to daughter cells that generate the distinct epithelial lineages of the small intestine – namely functional Paneth, absorptive, goblet, and enteroendocrine cells.[21] These processes are regulated by gene expression[22] and in health, asymmetric stem cell division provides a steady state, in which the stem cell compartment is maintained and the differentiated lineages are produced at rates equal to their losses.[23–26]

Regulation of enterocyte stem cells in vivo

Gastrointestinal stem cells respond to environmental influences and the dynamic equilibrium of the steady state may change. After bowel resection, crypt cell proliferative activity increases, resulting in increased functional enterocyte numbers, which are accommodated by an increase of villus height.[27 28] The converse occurs during prolonged starvation or bowel defunction.[29] Furthermore, the balance between stem cell self maintenance and provision of functional progeny may change, when mucosal losses are caused by stem cell injury or death. For example, stem cells are particularly sensitive to irradiation and most are killed after high sublethal doses. Sterilised crypts continue to empty of functional cells for a few days, then crypts disappear and bare ulcerated areas develop.[30] The surviving fraction of stem cells, which may be as low as 1/100 000 mount a proliferative response. Microcolonies, which consist of clumps of 10 or more cells, develop and assume a crypt outline, from the third and fourth day.[30 31] From the seventh day after radiation, the process of crypt budding or branching provides new crypts, each with its own stem cells.[32] With continued cell proliferation and development of new crypts, colonies enlarge, become visible to the naked eye, then ultimately coalesce to restore the mucosal layer. This neomucosa contains all normal intestinal cell lineages and persists for the life of the animal.[33] Similar responses are seen after cytotoxic agents are used.[34 35]

Hence, extensive mucosal losses may be restored in vivo, from very small stem cell numbers. The proliferation/differentiation balance of the steady state shifts towards stem cell renewal, resulting in increased stem cell number, genesis of new crypts, new cell lineages, and mucosal replacement. This phenomenon may be important in any application of enterocyte stem cell transplantation.

Influence of mesenchyme

Data concerning cellular or molecular stimuli that influence postnatal stem cell function in vitro, are scant. Proliferation and survival in culture of fetal endodermal cells, which have the pluripotent characteristics of stem cells, however, are enhanced by mesenchymal support. Endodermal cells, which have been cultured alone, generally fail to proliferate and only survive short intervals of up to four to five days. Those cocultured on a mesenchyme fibroblastic feeder layer, however, proliferate more readily, and can be maintained for longer intervals in vitro.[36]

During development, intestinal endodermal cells are responsive to mesenchymal stimuli by cell to cell and cell to matrix interactions. Epithelial maturation starts after development of close cell to cell contact, between surface epithelial cells and underlying mesenchymal cells.[37] Grafting experiments using avian embryonic intestinal endoderm, lead to well organised intestinal structures including crypt/villus architecture, only when combined with mesenchyme. Endoderm grafted alone fails to develop.[38] Recent work has shown that even a very small amount of mesenchyme grafted in combination with endoderm, will allow morphogenesis to

proceed in adult recipients. In these circumstances, however, additional mesenchyme support may be recruited from the tissue of the recipient animal.[39] Hence, it seems that epithelial/mesenchymal interaction supports proliferation of fetal endodermal cells in vitro and organotypic development after grafting in vivo.

Enterocyte culture and transplantation

Successful stem cell transplantation or autotransplantation requires the isolation and maintenance of stem cells ex vivo. In addition, the stem cells' capacity for organotypic development – that is, for restoration of cell lineages and tissue architecture, should be unaffected by the isolation, culture or transplantation procedures.

Most studies entailing isolation of intestinal epithelial cells in vitro and subsequent grafting in vivo have used intact undifferentiated fetal endoderm. Early endodermal cells are pluripotent, proliferate readily in culture, and in combination with mesenchyme, are capable of morphogenesis after transplantation.[36 39 40] Endoderm is not, however, representative of small intestine of later developmental stages. When the crypt/villus pattern becomes established in late fetal or neonatal intestine, proliferative stem cells are sparse and confined to crypt regions.[24-26] Hence, their isolation and culture is difficult[41] and functional villus cells, which are incapable of division, may predominate in cell isolations.

Data relating to morphogenesis by small bowel isolates of later development, are limited. Montgomery *et al*[42] isolated cells from 18 day fetal rat intestine by trypsin dissociation. Cells failed to proliferate in culture, which limits any potential for increasing cell number in vitro, but surviving cell clumps impregnated in gelatin squares showed development in vivo. Haffen *et al* showed morphogenesis by neonatal intestinal cells and embryonic mesenchyme, when placed as an interspecies recombinant graft into an embryonic environment.[43] This group also showed limited morphogenesis in 10% grafts of an immortalised postnatal epithelioid cell line [IEC-17],[44] when combined with fetal mesenchyme.[45] Successful isolation of normal intestinal proliferative cells, however, at late development stages, with proliferation and increase of cell number in vitro, then successful morphogenesis after transplantation into an adult environment, has not been reported.

An enzymatic method for isolation of small bowel epithelium and successful establishment of primary cell culture from the normal postnatal small intestine has recently been described.[46] This method has relied on disaggregation of intact crypt/villus units, which contain proliferative stem cells, functional epithelial cells, and mesenchymal tissue within the villus core. Hence, this method preserves the important epithelial/mesenchymal interactions during isolation and during the early stages of cell attachment and proliferation in vitro. When this method is used, cells attach readily and proliferate rapidly in vitro, to form coalescing colonies, with a 'cobblestone' appearance. Most cells develop brush border enzyme activity, express cytokeratins, and exhibit morphological features including epithelial tight junctions, desmosomes, and microvillus brush borders, which are characteristic of small bowel enterocytes.[46 47]

Grafting models were then developed to assess the capacity of cells isolated and cultured by this method, for organotypic development. In these studies, isolates were obtained from 20 day fetal rat intestine, in which the crypt/villus pattern is established. Isolates were maintained in vitro for four days then grafted in combination with fetal mesenchyme, to the renal subcapsular space of adult nude mice. On retrieval, grafts had showed progressive morphological development. At seven days after operation, a rudimentary lumen lined by a simple columnar epithelium was found, while by 14 days crypts, villi, and at least two small bowel epithelial cell lineages were present.[48] Hence, small intestinal epithelial stem cells may be isolated in mixed cell populations and successfully maintained ex vivo. Their capacity for organotypic development is retained after isolation and short term primary culture. Small bowel neomucosa may be generated, in ectopic sites of adult recipients, from grafts of these cell cultures.

Successful clinical application of a treatment entailing transplantation of small intestinal enterocyte stem cells would require a suitable recipient site in which peristalsis is maintained. We have shown that the colon provides a suitable site for small bowel mucosal autografts.[49] In that environment, small bowel morphology and function is maintained, after mucosal autotransplantation.[50] Hence, the colon is a potential recipient site for small bowel stem cell autotransplantation. Further experimental work, concerning cell lineage development, mesenchymal support, and motility is required, however, before the application of any of these methods to human tissues.

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