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LETTERS TO THE EDITOR

Vitiligo

Sir,

I read with much interest the two recent articles that appeared in Acta Derm Venereol (Stockh) 1993; 73: 49–51, entitled "Repigmentation of Vitiligo by Transplantation of Cultured Autologous Melanocytes" by Drs. Mats J. Olsson and Lennart Juhlin, and "Autotransplantation in Vitiligo: Treatment with Epidermal Grafts and Cultured Melanocytes" by Drs. Hugh Zachariae, Claus Zachariae, Bent Deleuran and Peter Kristensen. These papers reflect the importance of finding new therapies for vitiligo, since today's treatments are far from satisfactory. But, much has to be learned yet in regard to pathogenesis, before a definitive cure is found.

I would like to comment on two particular facts mentioned in these articles:

1. Although the area covered by minigrafts has a 1:1 ratio (donor area/recipient area), as pointed out by Drs. Olsson and Juhlin, when observing repigmentation by minigrafting, the ratio graft/yield is approximately 1:25, since melanocytes and pigment spread from the edge of the graft, up to 4–6 months post-grafting (1, 2). This implies that, for example, for every cm^2 of transplanted minigrafts, 25 cm^2 of achromic skin will become repigmented. Besides, to minimize scarring a 1 to 1.2 mm punch graft should be used. I also want to emphasize that minigrafting is the simplest method available today for repigmenting small to medium size areas of refractory and stable vitiligo.
2. Melanocytes, unlike fibroblasts and keratinocytes (the latter, if plated in high numbers) are not autonomous cells when grown in vitro. Fibroblasts grow independently from other cells, even at low platings. Keratinocytes, when seeded in low numbers, are very dependent cells, and need specific conditions in culture media with additional stimulation. Melanocytes are also very dependent cells, are more difficult to be grown adequately, and this is why in these two articles phorbol esters (Zachariae H. et al.) and basic fibroblast growth factor (Olsson M. et al.) were used to obtain appropriate melanocyte numbers for transplantation. Several questions arise at this point:

How safe is it to use potent cell stimulants for melanocytes without running the risk of future transformation of these cells? Phorbol esters are tumor promoters, and until proven absolutely harmless, it is perhaps safer not to use them for routine techniques in repigmenting patients with cultured cells. Basic fibroblast growth factor (3) and other wellknown melanocyte growth promoters in culture, such as pituitary extract, have not been shown to have deleterious effects when used in in vitro cultures. Nevertheless, keratinocytes have been demonstrated to regulate the number of melanocytes during embryogenesis (4), and also modulate melanocyte behavior in vitro (5), indicating that these cells are an important clue for regulation and control of pigment cells. With these facts in mind, could we say that cultured melanocytes are "normal cells" when grown without the regulatory influence of keratinocytes? Do they possess all the molecular information to continue their normal replication cycles in future cell generations? When senescence of melanocytes cultured in vitro occurs, during the several subculturing stages required for obtaining large numbers of cells that are appropriate for repigmenting vitiligo, are all cells completely "normal"? In other words, do melanocytes need the presence of keratinocytes, fibroblasts and other cells present under normal conditions in the skin to develop adequate regulatory mechanisms that could possibly prevent transformation of cells in culture?

Melanocytes, within epidermal sheets grown in vitro, may also give rise to some of these questions, although pigment cells under these circumstances are grown under more physiologic conditions (6), unless hormonal or molecular supplementation is added (7).

More needs to be known, before transplantation of melanocytes grown in culture is accepted as a routine, for a devastating disease such as vitiligo. The molecular basis of cell reproduction should be known more in depth before this occurs. Karyotyping could be a partial answer at this time, but this method does not cover the enormous population of cells generated during in vitro culturing. A single altered cell could originate an unwanted clone of transformed melanocytes.

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In response to the Letter by Falabella

We are grateful for the comments by Dr. Falabella. The bFGF used by us have, as Dr. Falabella points out, not shown any

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deleterious effects in culture. bFGF is a natural growth factor for melanocytes, produced in vivo by fibroblasts and keratinocytes and therefore probably an important factor of melanocyte behaviour and growth (1,2). It is normally present in nearly every tissue, bound to basement membrane and subendothelial cell extracellular matrix (3–5), and is probably liberated by any cellular or tissue injury. Local infusion and topical application of bFGF have been shown to enhance bone graft and wound healing, respectively (6). We found that melanocytes from adult and newborn skin, cultured in the presence of bFGF for a period of 3–8 weeks, always died after removal of bFGF, and they do not proliferate, unlike transformed cells. Not a single colony grew out from at least 400 million cells in the absence of growth factors. Similar long-time culture experiments have been reported by Lerner et al. (7). Nor could we see any negative morphologic changes in light microscopy such as multiple nuclei. As far as we know no one has been able to convert a normal pigment cell into a malignant one with the use of bFGF.

Dr. Falabella suggests that keratinocytes would be a more normal regulator of melanocytes. We have now in 40 recently transplanted patients used cultures with various percentages of keratinocytes and fibroblasts but have not seen any difference in the melanocyte growth or in the clinical healing effect.

As far as we know there is no theoretical or practical evidence that cultured cells initially stimulated by bFGF should be more prone to produce an unwanted clone than in the presence of keratinocytes.

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In reply to the letter by Dr. Falabella concerning our article on the treatment of vitiligo with epidermal grafts and cultured melanocytes, we would like to mention that we are prepared to study if the cultures can be performed without the use of phorbol esters, but we have to go through a substantial amount of more basic work before these conditions can be established. There are, however, at present no data, which indicate that phorbol esters in the concentrations we have used should be harmful (1). Concerning the influence of keratinocytes we do not want to discuss whether these cells are necessary for obtaining "normal melanocytes" in culture, but we can add that 75% of our cultures contain a variable amount of keratinocytes. The cells we have used for autotransplantation are also in general "young cells". The great majority are neither enlarged nor multi-nucleated. This does not guarantee that a single altered cell could originate an unwanted clone. This could, however, also happen with PUVA, the present standard therapy for vitiligo.

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