Is Thyroid Transplantation on the Distant Horizon?

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CORRECTING THYROID DEFICIENCY REMAINS a surprisingly controversial clinical area despite over 120 years having passed since thyroid hormone was first used therapeutically. George Murray used thyroid hormone therapy in 1891 in Newcastle upon Tyne, United Kingdom, and the treatment proved to have lasting success (1). Controversy surrounding the relative need for L-triiodothyronine (L-T₃) and levothyroxine (L-T₄) and the ratio in which they should, or should not, be prescribed has resulted in a large number of web sites that offer anecdotal support for a variety of opinions, leading to much confusion among patients (for one example, see www.stopthethyroidmadness.com).

Although the majority of hypothyroid patients feel well on L-T₄ therapy alone, there remains a small proportion who claim not to feel normal and require the addition of L-T₃ either as a separate tablet or as a mixture; usually in the form of desiccated thyroid. However, the use of L-T₃ by psychiatrists led to the opinion that most such patients were depressed (2), but these same cases forced physicians to ask whether they were indeed treating all of their patients appropriately. This uncertainty has only increased as a result of evidence suggesting that certain deiodinase type 2 polymorphisms (found predominantly in brain and pituitary), such as Thr92Ala, may be less efficient at converting thyroxine (T₄) to triiodothyronine (T₃) (3). Hence our continuing search for the ideal thyroid hormone replacement therapy.

The idea of solving such a problem by replacing the thyroid gland, no matter whether missing, dysfunctional, ablated, or removed, has been in circulation since Victor Horsley first suggested that grafting a portion of healthy thyroid gland would be a rational method of treatment (1); while this treatment was indeed initially successful, it was not long-lasting. With the obstacles to organ transplantation soon widely recognized, such ideas quickly dwindled. But with the advent of stem cell biology and the recognition that the normal thyroid gland harbors progenitor cells (4), the concept of thyroid cell replacement has once again become attractive.

Unfortunately, harvesting and culturing adult thyroid progenitor cells has been a formidable task, and alternative approaches to obtaining thyroid cells have been pursued. Much effort has been expended on nurturing embryonic stem cells into functional thyroid cells, and here there has been considerable success—at least in the mouse (4). But we should

backtrack to an earlier time in order to follow the progress in a logical fashion. An important observation was the realization that thyroid cells thrived when cultured in a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells (commercially called Matrigel). This mixture resembles the complex extracellular environment found in many tissues and includes a variety of growth factors such as insulin-like growth factor 1 (IGF1). Dispersed thyroid cells, inserted subcutaneously *in vivo* with this extracellular support, regrouped into thyroid neofollicles and secreted thyroglobulin, forming what was termed a thyroid “organoid” (Fig. 1) (5). Furthermore, research showed that such neofollicle formation was thyrotropin (TSH)-independent (6). However, mature thyroid cells have limited growth capacity, and such structures were unable to expand into a useful organ. The limited role of TSH in thyroid development was further emphasized by the TSH receptor knockout mouse (7,8). In this model, the thyroid actually formed in the right place but was small and markedly hypofunctional, illustrating that the major role for TSH was in thyroid growth and thyroid hormone synthesis and secretion. The pioneering work of Di Lauro demonstrated that it was the expression and regulatory interaction of transcription factors, in particular NK2 homeobox 1 (NKX2-1; previously called *TTF-1*) and paired box 8 (*Pax8*) joined with homeobox gene *Hhex* and *Foxe1* (previously called *TTF-2*), which formed a “thyroid specific gene expression program” responsible for development of the thyroid gland (9) and which interacted with a variety of additional regulatory factors such as *DREAM* (10). Subsequent studies with mouse embryonic stem cells (ESCs) demonstrated that thyroid progenitor cells expressing *Pax8*, a functional TSH receptor (TSHR), and the iodide transporter (NIS) could be obtained *in vitro* (Fig. 2) (11) and that IGF1 and insulin, as expected, were important in the induction of ESC thyroglobulin synthesis (12,13). While these studies were progressing there was another remarkable backdrop. In 2005, Altmann *et al.* (14) showed that a liver cell had the potential to be turned into a thyroid cell by transfecting *Pax8* and *NKX2-1* into Morris rat hepatoma cells as evidenced by thyroglobulin and thyroid peroxidase promoter/enhancer activation in their culture system. This gene combination, therefore, appeared to be the essential switching mechanism for thyroid cell differentiation and suggested that any cell could be programmed into a thyroid cell.

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FIG. 1. Histology of a human thyroid “organoid” 4 weeks post-transplantation into an immunodeficient mouse (hematoxylin and eosin staining, magnification $\times 400$). Reprinted with permission from Martin *et al.* (5).

FIG. 2. Murine embryonic stem cells (ESCs) expressing green fluorescent (GFP)-positive thyrotropin receptors (TSHRs) were cultured with activin A and formed embryoid bodies (A) with cells expressing *Pax8* and iodide transporter (NIS) as shown by reverse transcription polymerase chain reaction (B). Unexposed ESCs served as negative control and rat (FRTL-5) thyroid cells served as positive controls. Reprinted with permission from Ma *et al.* (11). Color images available online at www.liebertpub.com/thy

Antonica *et al.* (15) have now taken full advantage of this sequence of events, even if they failed to acknowledge it, by culturing murine ESCs in Matrigel and co-transfecting them with both *Pax8* and *NKX2-1* genes in the presence of TSH. They demonstrated most impressively that such cells could form functional thyroid follicles (Fig. 3) and that, after transplanting the differentiated cells into mice rendered hypothyroid by radioiodine ablation, functional thyroid tissue could develop. Indeed the cells developed so well that they corrected the induced hypothyroidism. This mouse model using stem cells transplanted beneath the kidney capsule, suggests that thyroid cell development and thyroid tissue formation can be expected to be successful in humans.

Another remarkable observation made in these studies was that transient expression of *NKX2-1* and *Pax8* was able to lead to long-term thyroid cell development of the ESCs. In other words, once these two genes were expressed for a time, the ESCs entered a thyroid cell development pathway that no longer required outside induction of these genes—“tickling” them was enough to get them moving, a phenomenon that requires more exploration.

Hence, at least in the mouse, thyroid stem cell development and transplantation is a reality. But the potential for immune attack on transplanted stem cells remains and so this direct approach would not be suitable for clinical application. The obvious answer to this problem will be to use the development of induced pluripotent stem (IPS) cells (16). This most important technical advance allows any cell to be reprogrammed into a pluripotent cell by the application of four genes, *OCT4*, *KLF4*, *SOX2*, and *cMYC* (16). These germline-competent cells can then be differentiated into the tissue-specific cell desired; in this case by the transfection of *Pax8* and *NKX1-2*. For example, dermal fibroblasts from a patient could be programmed into thyroid cells and introduced into the patient to re-form a thyroid organ. This approach would theoretically not induce a major destructive immune response, so it would appear highly feasible to provide patients

FIG. 3. Immunodetection of thyroglobulin (green) in a thyroid follicle derived from genetically engineered murine ESCs. Reprinted with permission from Antonica *et al.* (15). Color images available online at www.liebertpub.com/thy

with thyroid cell replacement therapy via the IPS approach. However, IPS cells may not be immunologically silent due to a number of variations in gene expression secondary to epigenetic differences and, furthermore, may retain the epigenetic memory of the tissue from which they are derived (17). So there is much more to learn in this exciting arena.

In summary, the possibility of thyroid cell therapy remains very attractive for patients with congenital hypothyroidism and for thyroid replacement after total thyroidectomy in patients with thyroid cancer. However, in patients with Hashimoto's thyroiditis or patients with Graves' disease after radioiodine or thyroidectomy, the immune system will no doubt also attack such transplanted cells unless the immune response has waned. And even if such immune reactivity has disappeared, the provision of new thyroid antigens may well reinitiate a lack of tolerance. In such patients, the transplanted cells would still need to be protected from immune attack—not such a simple task. And how safe would thyroid cell therapy be for those patients for whom it would be suitable? It would be hard to compete with cheap L-T₄ therapy. Furthermore, as mentioned earlier, differences in the epigenetic characteristics of IPS cells compared with ESCs have raised serious concerns about their potential abnormalities or even oncogenicity, but no doubt, these problems will be surmountable in time. What is for sure is that human thyroid transplantation is no longer just a dream but is on the horizon and very much a reality with considerable potential.

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