

Induced pluripotent stem cells and primary immunodeficiencies: A new frontier reached, a new world beyond?

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Key words: *Induced pluripotent stem cells, primary immunodeficiencies, gene therapy, disease pathogenesis*

Few articles in recent years have generated such interest and excitement as the report by Takahashi and Yamanaka¹ that adult stem cells could be reprogrammed to a pluripotent state. This study, closely followed by many others,^{2,3} demonstrated that cells from somatic tissue were not permanently differentiated and could, through the forced expression of specific transcription factors, be reprogrammed into cells that had all the transdifferentiation capacities of embryonic or other previously defined stem cell forms. The potential of these findings was immediately evident to not only stem cell biologists but also to scientists and physicians working in many diverse areas of medicine. The use of embryonic stem cells had already shown the enormous potential of stem cell medicine in allowing the generation of different tissues, organs, and eventually whole organisms⁴ but was still limited both technically and ethically by the reliance on embryonic tissue. Therefore the ability to take cell types, such as skin fibroblasts or, more recently, peripheral blood, and generate induced pluripotent stem (iPS) cells and then differentiate them into numerous other cell types provided a far more accessible and almost limitless mechanism to understand disease pathogenesis, screen for drug interventions, and develop regenerative or gene-modifying therapeutic strategies.

In this edition of the *Journal*, Pessach et al⁵ demonstrate that iPS cells can be generated for various different forms of immunodeficiencies. This in itself is not entirely novel because iPS cells generated from a patient with adenosine deaminase deficiency—severe combined immunodeficiency have been previously reported,⁶ and it is very likely that iPS cells from patients with other immunodeficiencies are currently being generated in many other laboratories. However, although this is the first formal report of this technique for a number of different forms of immunodeficiency, the importance of this article lies not in the technology or the rigor with which these lines have been characterized but in the possibilities that this technology holds for primary immunodeficiencies.

Initial benefits are readily apparent to those working in any aspect of translational medicine. Because of the rarity of primary immunodeficiencies, patient material is scarce. Murine models, cell lines, and small interfering RNA knockdown technology have a role, but all have their limitations, especially in their relevance to primary human disease.

Often material from patients with novel or unidentified gene defects is extremely limited because of the death of the patient or because, after hematopoietic stem cell transplantation, disease-specific hematopoietic tissue is unavailable. For example, take the investigation of a molecularly undefined patients with severe combined immunodeficiency many years after successful hematopoietic stem cell transplantation. It might be possible to make a genetic diagnosis by obtaining DNA from buccal swabs or skin fibroblasts, but if a novel gene defect is identified, it would be extremely difficult to understand the functional effects of the novel gene in lymphoid development without reverting to a knockout murine model or small interfering RNA knockdown of cell lines. Although the technology to successfully generate T or B cells from iPS cells is only now starting to emerge,⁷ it is very likely that in the above scenario it will be possible to generate an iPS line from skin fibroblasts and then to study the defect of mutant cells in lymphoid differentiation. Similarly, as the authors describe, it has been extremely difficult, to date, to understand the differential effects of diverse mutations in the same gene on lineage-specific cell fate and clinical phenotype. The use of iPS cells harboring such mutations and undergoing differentiation in similar conditions might allow us to understand how and where in development these differences arise.

Our ability to examine immune system pathogenesis is clearly enhanced, but the use of iPS cells to help understand the nonimmunologic abnormalities associated with specific primary immunodeficiencies is likely to be as important. A number of conditions, such as cartilage hair hypoplasia (bone and gut), DiGeorge syndrome (heart, thymus, and parathyroid among numerous others), and adenosine deaminase deficiency (skeletal, brain, liver, and auditory) have systemic manifestations as a result of widespread disease gene expression. Accessibility to disease tissue from patients has limited the understanding of the tissue-specific abnormalities, but the advent of iPS cells coupled with the rapid development of cell-culture protocols for differentiation to a diverse range of specific tissues now offers an opportunity to study these disease processes in far greater detail. It is also likely that in some cases this technology will allow us to find abnormalities in tissues that were previously thought to be unaffected by the disease.

Without doubt one of the greatest opportunities that iPS cell technology presents is for disease correction. Although for non-monogenic conditions cellular therapy through reprogramming or

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Disclosure of potential conflict of interest: H. B. Gaspar has received research funding from the Medical Research Council and the European Commission.

Received for publication February 22, 2011; accepted for publication February 23, 2011.

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J Allergy Clin Immunol 2011;■■■:■■■-■■■.

0091-6749/\$36.00

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doi:10.1016/j.jaci.2011.02.038

regeneration of autologous tissue might be possible, for primary immunodeficiencies, disease correction by iPS cells would necessarily have to be coupled with initial genetic modification of disease cells. The precedent for this has already been shown for other conditions. Using a knock-in murine model of sickle cell anemia, Hanna et al⁸ established iPS cells from tail-tip fibroblasts of the mice and subsequently corrected sickle globin genes using homologous recombination with a targeting construct containing the human wild-type globin gene. When hematopoietic progenitors arising from iPS cells were transplanted into irradiated sickle cell mice, all disease parameters of sickle cell anemia improved substantially. More recently, patient-specific iPS cells have been established and corrected by using viral gene transfer. Raya et al⁹ have reprogrammed dermal fibroblasts and keratinocytes of patients with Fanconi anemia to generate iPS cells, followed by genetic correction with lentiviral vectors encoding FANCA or FANCD2, by which the Fanconi anemia phenotype was corrected. Similarly, Tolar et al¹⁰ established iPS cells from keratinocytes and mesenchymal stromal cells from patients with mucopolysaccharidosis type I (Hurler syndrome), and after lentiviral transfer of the (α -L-iduronidase) *IDUA* gene, they were able to differentiate these cells into both hematopoietic and nonhematopoietic progenitor cell types. Importantly, transduced iPS cells have not shown any detectable differences in their differentiation ability compared with that of either human embryonic stem cells or normal iPS cells. iPS cells can also hold important advantages over gene therapy of adult stem cells. The ability to robustly preserve stem cell capacity over a prolonged culture period would allow for the analysis and selection of iPS clones that contained “safe” viral integration events, thereby overcoming the concerns regarding genotoxicity associated with current viral gene transfer strategies in adult stem cells.¹¹

Pessach et al⁵ envisage that a “novel frontier” for primary immunodeficiencies has been reached, and all in the field would hope that the land ahead is vast and plenty. However, it is important to ensure that what lies beyond does not turn into the wild west. As with any other cell and gene therapy strategies, it is paramount that the risks associated with iPS cells for therapy are

investigated in a detailed and rigorous manner before proceeding to clinical application in carefully controlled and regulated studies. Therefore it will be necessary to generate reproducible *in vitro* and *in vivo* models to investigate the potential risks of iPS cell-derived therapy, such as those related to genotoxicity of reprogramming with the specific transcription factors, using viral vectors for gene augmentation of mutated genes, and the risks associated with prolonged culture and acquisition and maintenance of the pluripotent phenotype and lineage-specific cell fates. These caveats aside, it is clear that iPS cells have a significant role to play in our further understanding and future treatment of primary immunodeficiencies.

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