Perspective
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Regenerative Medicine

Dendritic cells and pluripotency: unlikely allies in the pursuit of immunotherapy

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As the fulcrum on which the balance between the opposing forces of tolerance and immunity has been shown to pivot, dendritic cells (DC) hold significant promise for immune intervention in a variety of disease states. Here we discuss how the directed differentiation of human pluripotent stem cells may address many of the current obstacles to the use of monocyte-derived DC in immunotherapy, providing a novel source of previously inaccessible DC subsets and opportunities for their scale-up, quality control and genetic modification. Indeed, given that it is the immunological legacy DC leave behind that is of therapeutic value, rather than their persistence *per se*, we propose that immunotherapy should serve as an early target for the clinical application of pluripotent stem cells.

Keywords: cancer vaccination • dendritic cells • embryonic stem cells • genome editing • induced pluripotent stem cells • tolerance induction

Recent years have witnessed a growing appreciation of the role pluripotency may one day play in the design of novel approaches to the treatment of a broad spectrum of disease states requiring the replacement of individual cell types or tissues, compromised through trauma, disease or the natural process of aging. This realization culminated in 2012 in the award to John Gurdon and Shinya Yamanaka of the Nobel Prize for Physiology or Medicine for the work that led to induced pluripotency [1], thereby building on the earlier recognition in 2007 of Martin Evans' success in the isolation of mouse embryonic stem cells (ESC). This breakthrough not only made pluripotency accessible to experimental intervention *in vitro* [2], but paved the way for the later derivation of ESC from supernumerary human blastocysts [3] that has since heralded the era of regenerative medicine.

In parallel with these developments, the 2011 Nobel Prize was awarded to Ralph Steinman in recognition of the seminal work that led to the discovery of dendritic cells (DC) as professional antigen presenting cells (APCs), uniquely capable of initiating the immune response to foreign antigen by the activation of naïve T cells [4]. DC therefore provide unparalleled opportunities for intervening in the immune response at the point of its very genesis and thereby defining at source the outcome of antigen presentation. Nevertheless, various properties of DC limit their suitability for immune intervention: their limited lifespan and restricted capacity for expansion *ex vivo* have dictated the need for their differentiation from the peripheral blood monocytes of patients, even though such a source fails to recapitulate some of the properties of tissue-resident DC *in vivo*. Furthermore, such a source of DC has proven to be highly refractory to genetic modification, since the presence of heterologous nucleic acids is interpreted by DC as an indication of an immunological challenge by infectious microorganisms, inducing their terminal maturation. In contrast, pluripotent stem cell (PSC) lines, once generated, serve as a permanent resource, capable of indefinite self-renewal and expansion *in vitro*. As such, they offer ample opportunity for scale-up and quality control of the cell types differen-

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tiated from them, while proving to be relatively amenable to genetic modification through recent advances in genome editing [5]. Furthermore, by virtue of their pluripotency, they may serve as a potential source of functionally distinct DC subsets, capable of deploying the full therapeutic potential that DC have to offer. Here we discuss how harnessing PSC as an alternative source of DC, may help address many of the current obstacles to their use in a therapeutic context, paving the way for their future application to the treatment of a broad spectrum of unmet medical needs with an underlying immunological basis.

Dendritic cells: a complex network of functionally distinct subsets

As sentinels of the immune system, DC are widely distributed throughout both interstitial and lymphoid tissues where they serve to coordinate the response to infectious microorganisms. These they sense through expression of a broad repertoire of pattern recognition receptors (PRR), whose ligation by microbial products elicits their maturation into potent inducers of T-cell activation. The ability of DC to acquire and process protein antigens at the site of infection and present them to naïve T cells following their migration to the secondary lymphoid tissues, distinguishes them from all other APCs and sets in motion a series of carefully choreographed events that culminate in elimination of the causative agent of disease. Irrespective of their shared agenda, human DC represent a complex series of phenotypically and functionally distinct subsets whose lineage relationships have been subject to much speculation and debate [6]. Indeed, deficiencies in conventional systems of classification have recently inspired a new approach to their nomenclature based on their proposed ontogeny, as evidenced from similarities with their murine counterparts that are rather more amenable to investigation [7]. Although the debate concerning their classification is unlikely to be resolved in the near future, certain subsets of human DC are sufficiently well defined to allow their individual roles to be delineated, as illustrated by the coordinated response to a viral challenge at the mucosal surfaces or cornified epithelium of the skin.

Langerhans cells (LC), defined by their high expression of langerin (CD207), form a dense reticular network within the epithelium and therefore constitute the first line of defense against infection via this common route of entry. Unlike most other DC subsets, LC share with tissue macrophages the ability to trace their ancestry to embryonic precursors that colonize the developing skin *in utero* [8] and form a self-sustaining population that persists throughout life [9]. Their maturation in response to viral infection induces their migration to the draining lymph nodes, where they present processed antigen to $CD4^+$ MHC class II-restricted helper T cells (Th cells). Importantly, this subset also displays the capacity to 'cross-present' exogenous protein antigens to $CD8^+$ MHC class I-restricted T cells, which stimulates a cytotoxic T lymphocyte (CTL) response, essential for the elimination of virally infected cells. The capacity for antigen cross-presentation is shared by a trace population of DC resident in the secondary lymphoid tissues that are bone marrow derived and defined by their expression of CD141 and the chemokine receptor XCR1 [10,11] and which are, therefore, likewise critical for the clearance of viral infection by cell-mediated immunity. Should efforts to contain the infection fail, threatening the onset of viremia, precursors of so-called plasmacytoid DC in the peripheral blood respond through the secretion of exceptionally high concentrations of type I IFNs which help establish a global antiviral state that limits productive infection in tissues distant from the initial site of the challenge. Furthermore, these various populations of DC are supported in their function by circulating DC and monocytes that are actively deployed in response to the release of inflammatory mediators at the site of infection. *In situ*, recruited monocytes differentiate into inflammatory DC highly adapted to the acquisition, processing and presentation of exogenous viral antigens from liberated viral particles or through the phagocytosis of virally infected cells undergoing apoptosis. Although this population of inflammatory DC displays significant capacity for the activation of Th cells and the initiation of humoral immunity, they show very limited propensity for the cross-presentation of exogenous antigens, necessitating a reliance on other subsets for direct activation of the CTL repertoire.

Given that DC occupy such a critical place at the epicenter of the immune response to foreign antigen and thereby dictate both the nature and magnitude of subsequent events, they have proven to be attractive candidates for therapeutic intervention, inspiring numerous clinical trials over the past two decades. Nevertheless, the trace numbers and inaccessibility of many subsets of tissue-resident DC have dictated a dependence on the patient's own circulating DC or monocytes as a source of precursors that may be readily differentiated *in vitro* into DC displaying a pro-inflammatory phenotype. The reasons for focusing, almost exclusively, on this source of DC are largely pragmatic: unlike LC or $CD141^+$ DC from the secondary lymphoid organs, their abundance in peripheral blood makes them particularly accessible and amenable to production under clinically compliant conditions for re-administration to patients, a protocol that may be serially repeated in order to progressively

reinforce the immune response for vaccination purposes. Accordingly, monocyte-derived dendritic cells (moDC) pulsed with viral antigens, have been used for therapeutic vaccination to HIV-1 in an attempt to clear residual viral reservoirs [12,13] while DC differentiated from peripheral blood monocytes of cancer patients have been widely exploited for vaccination to defined tumor-associated antigens (TAA) [14–17]. Furthermore, by exposing moDC during the course of their differentiation *in vitro* to pharmacological agents such as rapamycin, 1α,25-dihydroxyvitamin D_3 (VD_3) or interleukin-10 (IL-10), it has proven possible to promote a tolerogenic phenotype [18], suggesting their potential applicability to scenarios requiring the establishment of antigen-specific tolerance [19], epitomized by Phase I trials aimed at the future treatment of Type 1 diabetes [20,21].

Despite their compelling credentials, the outcomes of clinical trials employing moDC, have been somewhat disappointing: indeed, during the 16 years since the first DC vaccination for melanoma [22], over 54 clinical trials have been conducted, a recent meta-analysis of which has revealed objective response rates of less than 10% [23,24]. This limited efficacy is further illustrated by the use of Provenge, the first DC-based therapy to receive US FDA approval for the treatment of metastatic hormone-resistant prostate cancer [25]. This product comprises autologous DC obtained by leukapheresis and cultured with a fusion protein between the TAA prostatic acid phosphatase and GM-CSF [26], the cytokine typically required for the generation of moDC. Re-administration of these cells to patients with two subsequent boosts, resulted in an increased median survival of 4.1 months compared with placebo. Such marginal benefits may be attributed, at least in part, to some of the vagaries of DC obtained either directly from the peripheral blood or through the differentiation of circulating monocytes, both of which display significant donor-to-donor variation both in the yield and quality of cells which may be further compounded by the disease state itself or its ongoing treatment. Monocytes are, for instance, susceptible to infection by HIV-1 which may undermine the functional integrity of DC differentiated from them [27], while long-term chemotherapy for the treatment of malignancy may adversely impact on bone marrow function on which circulating monocytes depend. Furthermore, the use of moDC is unlikely to be applicable to pediatric patients, from whom the low yield of cells would be likely to jeopardize the success of treatment. Most importantly, however, the functional phenotype of moDC is restricted by their limited capacity for cross-presentation of exogenous antigen to MHC class I-restricted CTL, a property essential for the cytolysis of malignant cells and subsequent reduction of the tumor burden. Together, such limitations have fueled attempts to identify alternative sources of DC whose properties may prove more conducive to their downstream clinical application, for which PSC are undoubtedly strong contenders.

Pluripotent stem cells: a novel source of DC

The first demonstration that PSC could be directed in their program of differentiation into fully functional DC made use of germline-competent mouse ESC as the starting material [28]. By first inducing the formation of embryoid bodies (EB), it proved feasible to guide differentiation along the mesoderm route and progressively restrict differentiation toward hematopoietic progenitors through the addition of GM-CSF and IL-3, from which terminally differentiated DC subsequently developed, displaying an immature phenotype [28]. Exposure of these cells to inflammatory stimuli or agonists of the PRR, promoted their terminal maturation into immunostimulatory DC, capable of initiating primary T-cell responses *in vitro*. Alternative protocols that avoided the EB stage, using, instead, the OP9 stromal cell line to initiate hematopoietic commitment, likewise proved successful, suggesting that multiple routes could be taken to navigate the DC lineage [29]. Recognizing their therapeutic potential, various laboratories adapted the original protocols for use with human ESC (hESC) [30,31], protocols that were subsequently optimized for downstream clinical use by the successive exclusion of animal-based products [32]. The advent of induced pluripotency in 2006 [1] galvanized efforts to replicate these early findings using induced pluripotent stem cells (iPSC) which proved successful, irrespective of whether the cells were of mouse (Figure 1A–C) or human origin (Figure 1D) [33,34]. This breakthrough raised the alluring possibility of producing DC from individual patients in a fully autologous manner, providing a welcome means of personalizing the therapeutic regimen which the use of moDC had previously offered, but ESC could never replicate [35,36].

Early studies of PSC-derived DC revealed various advantages over conventional populations, including their reproducibility and potential for expansion and scale-up *in vitro* [37,38]. The power of pluripotency to deliver potentially unlimited numbers of cells for therapeutic purposes, was amply illustrated in the mouse, in which individual EB were shown to generate in excess of 2 x 10^7 DC on several successive occasions before eventually reaching senescence [38]. Although human cells have undoubtedly proven more challenging in this respect, differentiation protocols are likely to be amenable to further optimization of the manufacturing

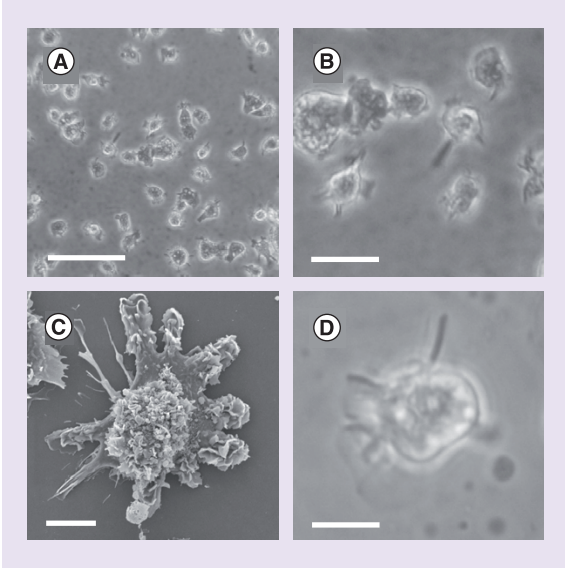

Figure 1. Morphology of dendritic cells differentiated from induced pluripotent stem cells. Photomicrographs of dendritic cells differentiated from **(A–C)** mouse- and **(D)** human-induced pluripotent stem cells viewed under **(A, B & D)** phase contrast and **(C)** scanning electron microscopy. Scale bars represent: **(A)** 50 µm **(B)** 20 µm **(C)** 5 µm and **(D)** 10 µm.

process in order to ensure yields of DC that are fully conducive to downstream clinical applications. The opportunity for scale-up is, however, especially important when combined with the potential that PSC provide for the differentiation of functionally distinct DC subsets, many of which are present in negligible numbers in their tissues of origin and whose therapeutic value therefore remains untested. Whereas PSC-derived DC differentiated via the original protocols display a gene expression profile similar to conventional moDC [39], the addition of GM-CSF, TGF-β and TNF-α to cultures of hematopoietic progenitors derived from either hESC or human iPSC (hiPSC) has succeeded in directing their differentiation towards populations of *bona fide* CD207$^+$ LC [40]. These findings suggest that, by virtue of their pluripotency, ESC and iPSC may provide opportunities to recapitulate populations of cells derived *in utero* from embryonic progenitors that would not normally be accessible through the culture of bone marrow-derived cells. Furthermore, hiPSC have been coaxed to differentiate into DC expressing CD141 and XCR1 (Figure 1D), which were shown to cross-present exogenous protein antigen to a CD8$^+$ CTL clone and naïve peripheral blood T cells [34], thereby providing a novel source of a subset of DC dedicated to the cross-presentation of exogenous antigen, otherwise found in trace numbers in the peripheral blood and secondary lymphoid tissues [10,11,41,42]. These findings amply illustrate the potential that pluripotency offers for accessing even the rarest of cell types in numbers sufficient to probe their lineage relationships, function and therapeutic potential. Furthermore, it may prove feasible to answer critical questions concerning their basic biology through genetic modification of the parent PSC which are far more tractable for such purposes than fully differentiated DC.

Exploiting pluripotency for the production of 'designer' DC

The heightened sensitivity of DC *in vivo* to the presence of infectious microorganisms causes them to actively resist the introduction of heterologous DNA, to which they vigorously respond by the acquisition of a mature, proinflammatory phenotype, associated with a limited life-span. Consequently, conventional approaches to genetic modification, such as electroporation, lipofection or the use of cationic peptides, have typically enjoyed only limited success [43]. The feasibility of introducing transgenes of interest into the parent PSC line from which DC expressing the desired mutant phenotype may be subsequently differentiated was first demonstrated using mouse ESC [44]. The use of a suitable reporter gene revealed the relative ease with which an appropriate clone of ESC could be produced that might serve as a permanent resource amenable to cryopreservation and from which DC could be differentiated, uniformly expressing the desired mutant phenotype. This phenotype was shown to be highly reproducible between successive batches of DC [37] which, most importantly, were found to retain an immature phenotype unadulterated by the introduction of the transgene: indeed, the genetic modification of the parent cell line was found to have no discernible impact on either the phenotype or function of the DC which acquired potent immunostimulatory capacity upon subsequent maturation and migrated to the draining lymph nodes upon re-administration to recipients [44].

This approach to the genetic modification of DC has been widely exploited over the past few years. In particular, it has proven an attractive way of introducing target antigens into DC to which immune responses might be desirable, their endogenous expression permitting presentation via MHC class I to CD8$^+$ T cells, irrespective of the propensity of the resulting DC for antigen cross-presentation. For example, Motomura and colleagues expressed in mouse ESC the murine homolog of glypican-3, an oncofetal antigen expressed by human melanoma and hepatocellular carcinoma [45]. DC differentiated from the genetically modified cell line were able to prime CTL responses to the TAA *in vivo* which protected against a subsequent challenge with the B16-F10 tumor cell line, naturally expressing glypican-3. That such an approach

might also enhance the functional capacity of the DC themselves, or even confer on them additional properties of therapeutic value, was elegantly demonstrated by the simultaneous expression of a surrogate tumor antigen and specific chemokines, known to promote the migration of DC to the secondary lymphoid tissues. Importantly, DC differentiated from double-transfected ESC proved more effective at promoting protective tumor immunity than those differentiated from ESC expressing the tumor antigen alone [46].

In principle, a similar approach might be used to introduce target antigens and other genes of interest into DC differentiated from ESC and iPSC of human origin, a possibility elegantly demonstrated by Senju and colleagues [39]. Expression of a version of the human invariant chain, engineered to contain an epitope of GAD65 within the class II-binding region, permitted the differentiation of DC capable of constitutively presenting the epitope to an antigen-specific Th-cell clone. Although this study employed traditional approaches to genetic modification using plasmid DNA, the recent advent of the CRISPR/Cas9 system offers a powerful new technique for genome editing [47,48] fully amenable to use with hiPSC [5], that will undoubtedly open up new horizons for the rational design of DC for use in immunotherapy. In particular, such an approach allows the introduction or correction of specific mutations with great precision at defined locations in the genome while incurring minimal off-target effects, an achievement previously inconceivable with strategies based solely on homologous recombination. Although pragmatic issues may limit the application of genome editing to personalized therapies, the approach is likely to find application in the identification of molecular targets for additional intervention in order to maximize the impact of immunotherapy. For instance, forced expression of PD-L1 by hESC-derived DC has been shown to significantly reduce T-cell responses to antigen [39], suggesting that disruption of the PD-1/PD-L1 axis may enhance the immune response to TAA. Given that PD-1-specific mAb have already proven effective in the treatment of advanced melanoma [49,50], such findings may provide a compelling rationale for their use in future combination therapies, for which DC-based vaccines may provide the required antigen specificity.

PSC-derived DC for vaccination purposes

Given the acknowledged limitations to the use of moDC in strategies for immunotherapy, many of which may be circumvented by harnessing the properties of pluripotency, much interest has focused on the use of DC differentiated from pluripotent source material for vaccination purposes, most studies to date having explored their utility for enhancing tumor immunity. Indeed, plans for the use of hESC in such a scenario are already well advanced, the pharmaceutical company Asterias having recently announced the imminent recruitment of patients to a Phase I clinical trial for the treatment of non-small-cell lung cancer [76], which will doubtless serve as a valuable test case for the design of all future trials. There are persuasive pragmatic reasons for the use of ESC rather than iPSC for such a ground-breaking trial, since numerous ESC lines are now available that are not only GMP compliant but fully characterized, providing an off-the-shelf product in a far more timely manner and at a fraction of the cost required for the derivation of patient-specific iPSC lines. Nevertheless, second-generation vaccines based on the use of ESC will necessarily be subject to various constraints by virtue of their allogeneic origin. The absolute requirement for MHC class I-restricted antigen recognition by CTL in order to target a tumor mass will, for instance, dictate the need for matching at one or more MHC class I loci, thereby greatly restricting the pool of patients that may benefit. More importantly, however, disparities at the remaining MHC loci will inevitably elicit potent alloreactivity within the T-cell repertoire of the recipient which may serve to dilute the antitumor response and provoke the demise of the administered DC, both through lysis by recipient CTL [51] and activation of natural killer (NK) cells [52].

Although this is certainly a significant complication, studies in mice suggest that, paradoxically, the use of semi-allogeneic DC need not impede their capacity to elicit an antitumor response [53] and may even prove beneficial in some circumstances, since widespread alloreactivity may provide a much-needed adjuvant effect. Indeed, alloreactivity may create a global, pro-inflammatory environment capable of breaching the natural tolerance to TAA which are, by definition, self-antigens to which immune responses are normally curtailed by the activity of regulatory T cells (Treg) [54]. Nevertheless, the opposing forces of adjuvanticity and alloreactivity are likely to be finely balanced, provoking an unseemly race to establish a tumor-specific response before the elimination of the administered cells, the outcome of which will depend wholly on the nature and magnitude of the MHC disparities between donor and recipient, introducing an unwelcome element of unpredictability into any treatment regime.

In order to influence the outcome of such a race, and reduce the level of uncertainty, Zeng and colleagues genetically modified hESC to express CD1d, a nonconventional MHC class I molecule that presents glycolipids to invariant NKT cells (iNKT cells) [55]. DC differentiated from them were shown to efficiently recruit and expand human iNKT cells

in vitro through presentation of α-galactosylceramide and to elicit a potent proinflammatory cytokine profile that favored priming of semi-allogeneic $CD8^+$ T cells to the melanoma-derived antigen, MART-1, presented by the same DC via MHC class I [55]. Using a rather different approach in a mouse tumor model, Fukuma and colleagues overexpressed in ESC the gene encoding SPI-6, a specific inhibitor of granzyme B, that renders cells less vulnerable to CTL lysis. Accordingly, administration of genetically modified DC to semi-allogeneic recipients led to an enhanced capacity to prime TAA-specific responses by extending their life span *in vivo* [53]. Notwithstanding such encouraging findings, there is little doubt that DC will survive only transiently in semi-allogeneic recipients and that their elimination will establish immunological memory likely to prove a formidable barrier to subsequent administration of the same source of cells and greatly limiting opportunities to progressively augment immunity through successive inocula. Indeed, Hermans and colleagues showed that antitumor immunity was limited by the rapid clearance of syngeneic antigen-laden DC when administered to mice previously primed with the same antigen [56], implying that a semi-allogeneic source would suffer an even more ignominious demise. Such studies suggest the need to re-evaluate the use of patient-specific iPSC in such a context in order to limit any confounding responses that may detract from the establishment of antitumor immunity.

While the capacity to derive iPSC from individuals in an autologous manner is undoubtedly the property most likely to define them as the preferred source of the next generation of DC vaccines, there is little doubt that the economics of personalized medicines are currently far from favorable. Nevertheless, experience from the marketing of Provenge, which cost US $93,000 when first launched in 2010 [25], would seem to imply that the market for personalized immunotherapies is surprisingly undiscerning as far as cost is concerned. Given that ample opportunities exist for streamlining both the production of iPSC lines and their differentiation *in vitro*, there is little doubt that substantial reductions in cost will prove feasible to attain in future. Irrespective of ongoing debates surrounding the health economics of personalized medicines, however, the scientific case for the use of autologous iPSC for DC-based immunotherapy is rather more persuasive.

First, the feasibility of deriving iPSC from a variety of somatic cell types such as dermal fibroblasts, ensures that their production is unimpaired either by the disease process itself or its ongoing treatment, unlike bone marrow-derived monocytes which may be adversely affected by long-term chemotherapy. Second, the demonstration that hiPSC may be differentiated into the previously inaccessible $CD141^+XCR1^+$ subset of DC, offers opportunities to exploit the cross-presentation of exogenous TAA direct to MHC class I-restricted CTL [34], a property that is essential for effective tumor eradication but which is poorly developed among hESC-derived DC whose gene expression profile is most closely allied to moDC [39]. The capacity for cross-presentation permits target antigens to be introduced in the form of recombinant proteins from which appropriate class I and class II restricted epitopes may be selected by DC during antigen processing, thereby obviating the need to genetically modify the parent cell line in order to endogenously express the relevant genes [31,32,39]. Most importantly, however, the potential for deriving iPSC in an autologous manner avoids alloreactivity and the establishment of immunological memory that would limit the repeated administration of the DC they spawn, necessary to progressively augment immunity over time in a 'prime-boost' fashion.

PSC-derived DC for the induction of tolerance

Although the scientific literature is rife with reports of the capacity of pharmacological agents such as rapamycin,

VD_3 and IL-10 to render moDC more tolerogenic [19], their exploitation for the induction of antigen-specific tolerance remains in its infancy, most clinical trials having demonstrated their safety profile *in vivo* but, as yet, little evidence of efficacy [20,21]. Nevertheless, there are some intriguing indications that DC differentiated from PSC may lend themselves to such indications better than their monocyte-derived counterparts.

One of the enduring questions surrounding the ability of PSC to generate a variety of hematopoietic cells *in vitro* is whether the resulting cell types are the products of primitive or definitive hematopoiesis [36,57]. Although recent studies have demonstrated unequivocally the capacity of hESC and hiPSC to support definitive hematopoiesis, as evidenced by their ability to sustain T-cell development [58], true hematopoietic stem cells (HSC) derive from a population expressing a $KDR^+CD235a^-$ phenotype that may be produced *in vitro* only through manipulation of Wnt-β-catenin signaling [59], the default pathway of differentiation following a more primitive route that bypasses the need for HSC. Current protocols for the differentiation of DC would appear, therefore, to generate cells of an embryonic or fetal origin, circumstantial evidence in support of such a notion coming from reports of the derivation of cell types, such as LC, known to develop from embryonic progenitors rather than bone marrow-derived HSC [40]. An important corollary to such findings is, however, that DC of fetal origin are inherently protolerogenic [60] in order to help maintain a state of fetomaternal tolerance during the course of gestation, the loss of which might otherwise induce spontaneous abortion. This phenotype is characterized by low expression of MHC class II and co-stimulatory molecules, such as CD40 [61], but is especially evident at the level of secretion of IL-12, a proinflammatory cytokine required for the initiation of Th1 responses and CTL activation [62]. Goriely and colleagues showed the molecular basis of this deficiency to be active repression of the gene encoding the p35 subunit of IL-12 which could be partially reversed through IFN-γ signaling [61,63].

Interestingly, DC differentiated from PSC display characteristics consistent with their unconventional provenance, likewise indicative of a more tolerogenic phenotype. Gene expression profiling of DC differentiated from mouse ESC revealed a high degree of identity with bone marrow-derived DC treated with VD_3 [37], such treatment having been shown to render them potently tolerogenic, as evidenced by their ability to induce dominant tolerance to skin grafts mismatched at minor histocompatibility loci when administered to recipient mice [64]. These findings are consistent with observations of DC differentiated from hESC and hiPSC which constitutively express both MHC class II and co-stimulatory molecules at lower levels than moDC [39] and require activation via a complex cocktail of proinflammatory cytokines and agonists of the PRR in order to secrete bioactive IL-12. In contrast, PSC-derived DC produce high levels of IL-10, an anti-inflammatory cytokine known to contribute to the establishment of tolerance through the induction of Treg cells [65].

In order to explore the potential utility of PSC-derived DC for the induction of antigen-specific tolerance, Hirata and colleagues serially transfected mouse ESC with an epitope from the CNS autoantigen, myelin oligodendrocyte glycoprotein (MOG), together with the inhibitory receptors PD-L1 and TRAIL. When administered to mice susceptible to experimental autoimmune encephalomyelitis (EAE), the resulting DC were found to significantly impair T-cell responses to MOG, an outcome that was accompanied by a decrease in T-cell infiltration within the CNS and a commensurate reduction in severity of EAE [66]. Interestingly, the beneficial effects of this treatment regime were found to be mediated primarily through the induction of Treg cells, since their ablation with mAb specific for CD25 significantly diminished the therapeutic benefit [67].

Although such studies provide a welcome proof of principle for the exploitation of PSC-derived DC in contexts other than vaccination, their use for the treatment of ongoing autoimmune disease is undoubtedly an ambitious goal, requiring the re-establishment of tolerance in a primed immune system. Nevertheless, various disease states, such as hemophilia and the lysosomal storage diseases, are caused by the loss of a defined gene product, the replacement of which with recombinant protein or through gene therapy would likely prove restorative, were it not for a neutralizing immune response that limits the effectiveness of the approach. Under such circumstances, the induction of tolerance to the required gene product in advance of its administration would greatly facilitate subsequent treatment [68–70]. Given the early onset and presentation of such conditions, the use of DC differentiated from iPSC might provide a rational approach to the induction of antigen-specific tolerance, since the use of hESC would be precluded by virtue of their allogeneic origin. While it remains to be seen whether disparities at specific MHC loci will provide useful adjuvanticity to promote antitumor immunity, there is no doubt that such alloreactivity would confound any attempts to induce tolerance, the proinflammatory environment caused by the administration of allogeneic DC proving potently antagonistic to such ambitions. Although under such circumstances the use of an autologous

source of DC would be essential, it is highly unlikely that sufficient monocytes or peripheral blood DC could be obtained from most pediatric patients for such purposes: the production of autologous iPSC lines, while not without its complexities, might, therefore, provide the necessary means to scale-up the production of DC in a manner conducive to their downstream use for immune intervention.

Immunotherapy: an early target for the use of PSC?

Ever since the publication in 1998 of James Thomson's seminal paper describing the derivation of ESC from supernumerary human blastocysts [3], there has been much speculation concerning their potential use in cell replacement therapy for the treatment of a wide variety of disease states [71]. Furthermore, the subsequent description of induced pluripotency [1] has done little to assuage public enthusiasm for such advances. Nevertheless, progress to the clinic has been slow, with spinal cord injury, Stargardt's macular dystrophy and age-related macular degeneration [72,73] remaining the only indications in which the differentiated products of PSC have so far been trialed in patients. The reasons for such a slow rate of progress are manifold, not least of which is the requirement for precise delivery of the cells to the site of the lesion as well as their functional integration and indefinite survival. This is paramount despite the hostile environment afforded by most lesions which are typically proinflammatory, hypoxic and devoid of nutrients. Furthermore, there are significant safety issues that still need to be addressed, associated with the stability of the differentiated phenotype and the potential for tumorigenesis [74,75], either through the direct acquisition of a malignant phenotype by the differentiated cells or the carryover of residual PSC within the inoculum. The risks involved are undoubtedly exacerbated by traditional approaches to the generation of iPSC requiring the stable integration of pluripotency genes that carries with it a high probability of insertional mutagenesis.

Given the protracted time frame over which these obstacles will need to be systematically addressed in order to facilitate successful translation to the clinic, we propose that the use of PSC as a platform for DC-based immunotherapy may constitute a rational early target for their clinical application. The main reason for focusing on immunotherapy in this context is that the success of immune intervention is not dependent on the indefinite survival of the products of differentiation but rather the immunological legacy they leave behind: providing administered DC have productively interacted with the T-cell repertoire, their subsequent demise poses little threat to the long-term success of the treatment regime and is, instead, to be welcomed. Since the normal life-span of DC following maturation is restricted to a few days, cellular material derived from PSC will be rapidly eliminated following administration, leaving no trace of the cells of origin. Indeed, the only lasting evidence of their transient presence is likely to be found within the T-cell repertoire of the recipient which will now contain the desired antigen specificities in far greater abundance, either in the form of effector CTL in the case of cancer immunotherapy or Treg cells, should tolerance be desirable. Such rapid clearance of the administered cells should reduce the likelihood of adverse reactions, including any propensity for tumorigenicity. Although there have so far been no reports of teratoma formation in any of the animal studies involving engraftment of hematopoietic cells derived from hESC [36], a further safety margin may be introduced into any prospective trials by exposing the fully-differentiated DC to ionizing radiation. Such a treatment regime, has been shown to have little impact on their functional potential [32] but would be likely to prevent the establishment of tumors in the event that residual PSC had evaded normal quality control and been erroneously administered to patients.

In addition to such a compelling safety profile, experience shows that DC may be administered via various routes due to their capacity to migrate from the site of injection to the draining lymph nodes in response to chemokine gradients [44]. Accordingly, subcutaneous, intradermal and intravenous injection of moDC have all proven to be safe and effective in trials of vaccination to melanoma antigens [24]. Furthermore, the ability to establish immunity over a period of time through the administration of DC on successive occasions, suggests that the numbers required for each inoculum are already within scope of current protocols, although these will undoubtedly benefit from further refinement and streamlining of the manufacturing process. We would argue, therefore, that these substantial advantages, together with the broad spectrum of disease states that may benefit from immune intervention, make DC-based immunotherapy the 'low-hanging fruit' of induced pluripotency.

Conclusion

Clinical trials conducted over the past two decades have taught us that strategies for immunotherapy based on the use of DC are both safe and effective for certain indications but that moDC may not constitute the optimum population for use in this context since they have at their disposal only a fraction of the therapeutic potential displayed by other subsets. The availability of hESC lines and the advent of induced

pluripotency have, however, broadened our horizons, providing access to alternative subsets of DC whose therapeutic potential has yet to be formally tested while offering prospects for genome editing that may enable us to gain a better understanding of their basic biology and function. It is by seizing the opportunities that pluripotency has to offer that a new generation of DC-based therapies may emerge for the future treatment of a wide variety of disease states.

Future perspective

The exploitation of pluripotency for the purpose of immunotherapy is poised to enter an exciting phase as plans for clinical trials of hESC-derived DC for cancer vaccination approach fruition. The results of these ground-breaking studies will provide essential information about the safety and efficacy of such an approach that will undoubtedly galvanize the therapeutic use of DC differentiated not only from hESC but from alternative sources including iPSC, tailored to the needs of individual patients. Although the health economics of such personalized medicines remains challenging, global efforts to streamline and automate the derivation and differentiation of iPSC and a growing consensus as to the most appropriate reprogramming methodologies suggest a future in which both the costs and timelines involved are conducive to downstream clinical application. The likely progression to hiPSC, will not only provide a rational means of addressing the issues of alloantigenicity that confound the use of hESC but may facilitate their use for the treatment of numerous disease states requiring the induction of tolerance, greatly increasing the indications for which DC-based immunotherapy may prove beneficial. While the fetal phenotype of DC differentiated from this novel source resonates with such ambitions, the recent identification of the signals responsible for promoting definitive hematopoiesis from PSC may provide access to many other DC subsets of which our understanding is currently limited but whose therapeutic value remains untested. Prospects for the differentiation of the DC subsets

Executive summary

Dendritic cells as agents for immune intervention

- Dendritic cells (DC) serve as professional antigen presenting cells (APCs) required to initiate the immune response to foreign antigen and maintain a state of tolerance to self-components.
- The capacity to derive DC from peripheral blood monocytes of individuals has permitted their use in strategies for immune intervention.
- Monocyte-derived dendritic cells (moDC) have been widely exploited for vaccination to tumor-associated antigen and infectious microorganisms such as HIV-1, but their use for the induction of antigen-specific tolerance remains largely untested.

Limitations of moDC

- Although some trials of moDC have yielded encouraging results, objective response rates have been largely disappointing.
- moDC show significant patient-to-patient variation in quality and yield which may be further compounded either by the disease itself or its ongoing treatment.
- moDC display only limited functional potential including only limited capacity for the cross-presentation of exogenous antigen to the cytotoxic T lymphocyte repertoire.

Pluripotent stem cells as an alternative source of dendritic cells for therapeutic purposes

- The availability of GMP-compliant human embryonic stem cells (hESC) and the advent of induced pluripotent stem cells (iPSC) has inspired the development of protocols for their differentiation along the DC lineage.
- Exploiting pluripotency may provide access to rare subsets of DC whose therapeutic potential has yet to be tested.
- Pluripotent stem cell (PSC)-derived DC show greater consistency between batches than moDC and their unconventional origin offers opportunities for scale-up, quality control and genetic modification through emerging technologies for genome editing.
- Since PSC-derived DC appear to emulate DC of fetal origin, their phenotype is inherently more tolerogenic, suggesting their use not only for vaccination purposes but also for the induction of antigen-specific tolerance.

Immunotherapy as an early clinical target for PSC

- The use of PSC for cell replacement therapy faces many obstacles associated with the functional integration of differentiated cell types and their long-term survival in the absence of tumorigenicity.
- The success of immunotherapy relies on the immunological legacy left by DC rather than their persistence *in vivo*.
- PSC-derived DC have a short life-span following their maturation: their use in strategies for immunotherapy may carry minimal risks of tumorigenicity, making them an attractive early target for the clinical application of PSC.

most appropriate to the immunological challenge offer a level of fine control that has not previously been feasible but may one day prove within reach.

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